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LIFE HISTORY OF LUNGWORMS PARASITIC IN SWINE

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INTRODUCTION

The adult nematodes parasitic in the lungs of swine comprise 2 closely related genera, *Metastrongylus* and *Choerostrongylus*, and 3 species, namely, *M. elongatus*, *M. salmi*, and *C. pudendotectus*. These nematodes belong to the family Metastrongylidae, the members of which, so far as is known, are parasitic in the respiratory and circulatory systems of mammals. The metastrongyles which parasitize domesticated animals are of considerable medical interest, and of unquestionable economic importance. They present for solution problems in livestock sanitation which must await the results of further investigations elucidating the life histories of the species concerned. Rational control measures for these, as well as for all other zooparasites, must be based on precise information regarding essential facts in the life histories. Such information for swine lungworms is presented in this bulletin.

REVIEW OF WORK OF PREVIOUS INVESTIGATORS

Up to 1929 little was known of the life history of the species of lungworms which occur in domestic swine. Leuckart (*8*, *v. 2*)[1] in 1876 expressed the opinion that the species of swine lungworm now known as *Metastrongylus elongatus* was a heteroxenous nematode, having its intermediate stage in an invertebrate, probably an insect or a snail. According to Alessandrini (*1*, *p. 479*), Cobbold shared Leuckart's view with regard to the heteroxenous nature of swine lungworms and considered earthworms as the probable intermediate hosts. The following is a rather free translation of the statement made by Alessandrini.

> If the supposition of Cobbold can be confirmed, the life cycle would be very simple; the eggs and larvae that are eliminated while the sick animals are coughing, would reach the ground, where they would be engulfed by earthworms. In the stomach, intestine, or tissues of these worms the larvae would continue their development and, eliminated to the outside with the excreta of earthworms, could infest other animals.

However, neither Leuckart nor Cobbold presented proof to substantiate his hypothesis. Work carried out in the Zoological Division of this Bureau by Ransom, as reported by Mohler in 1920 (*11*), and by Schwartz in 1921[2] independently of each other, showed fairly conclusively that lungworms failed to develop in swine when the embryonated eggs or the larvae which hatch in vitro from the eggs taken from the uterus were administered to presumably susceptible pigs, observations which were confirmed later by Hobmaier and Hobmaier (*5*). These results pointed to the likelihood of an intermediate host being involved in the life cycle of these parasites.

Von Linden and Zenneck (*9*) in 1915 and Zebrowski (*15*, *16*) in 1922 and 1925 reported that swine lungworms had a life cycle which did not involve an intermediate host. Von Linden and Zenneck investigated the life histories of several species of lungworms, including one of the species which occurs in swine, this species being designated by these investigators as *Strongylus paradoxus*, a name which is now regarded as a synonym of *Metastrongylus elongatus*. Whether these investigators actually worked with *M. elongatus* or with one of the other species of swine lungworms, or with a mixture of species, cannot be determined from data given in their paper. They report, however, that the eggs and larvae of lungworms obtained from wild hogs developed in sterilized soil into free-living individuals showing sex differentiation, and that these maintained themselves as free-living nematodes for several generations.

An examination of Von Linden and Zenneck's illustrations shows that whereas those of the egg and first-stage larva are unquestionably figures of a species of swine lungworm, those of the supposed free-living generation are figures of nematodes not known to be parasitic in animals and involve apparently more than one species; one of the species in question appears to belong to the genus *Rhabditis*.

It is clear that Zebrowski also was dealing at least in part with larvae of free-living nematodes, a supposition well supported by an

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[1] Italic numbers in parentheses refer to Literature Cited, p. 40.
[2] Unpublished data.

examination of his figures and by his account of the free-living stages of the supposed parasites. It is interesting to note, however, that Zebrowski (*15*) considered the possibility that swine lungworms might require an intermediate host and that he even exposed earthworms to an experimental infection with "larvae in different stages of development." His results were inconclusive but he found nematode larvae in earthworms.

In 1929 Hobmaier and Hobmaier (*5*, *6*) published the results of their investigations on the life history of two species of swine lungworms, *Metastrongylus elongatus* and *C. pudendotectus*, and incriminated earthworms, species not well defined,[3] as intermediate hosts. Briefly, these investigators noted one molt in the course of the development of the larvae in the wall of the esophagus and in the hearts of earthworms, the infective stage being attained following this molt. They demonstrated, moreover, that pigs could be experimentally infected with these two species as a result of ingesting earthworms harboring the infective stage of the parasites. These investigators noted also only one molt in the course of the development of lungworms in the definitive host. Their work, so far as the mode of transmission of lungworms to swine was concerned, practically fulfilled all the requirements essential to the demonstration of the life cycle of a heteroxenous nematode.

The transmission of swine lungworms by earthworms was later confirmed by the present writers (*13*), who observed two molts in the course of the development of the larvae in the intermediate host. Though the writers' results with reference to the number of molts were subsequently questioned by Von Schuckmann and Zunker (*12*), who confirmed the work of the Hobmaiers, a brief paper which embodied the results of the writers' investigations (*14*) on this subject up to the middle of 1931 established conclusively the facts with regard to the number of molts in the course of development of the two species of lungworms under discussion.

TECHNIC USED IN INVESTIGATION

Adult lungworms were obtained from the bronchioles of lungs of swine from various parts of the country. The worms were carefully removed with the aid of forceps, washed in physiologic saline solution, and sorted as to species. When infested with lungworms, swine almost invariably harbor a mixed infestation with *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*, and are rarely infested with *M. salmi*. The intensity of infestation with each of the three species was in the order in which these species have been named.

The gravid, intact females were cut into small pieces with a pair of fine scissors in a glass dish containing a small quantity of water. The cut-up material usually contained a mixture of thin-shelled eggs and larvae which had just emerged from the eggs, as well as thick-shelled embryonated eggs; the latter did not hatch in vitro. This material also contained nonembryonated and incompletely embryonated eggs which degenerated quickly in water and in physio-

[3] These investigators referred to the earthworms used in their investigations as *Lumbricus terrestris* major and minor, varieties which have no zoological standing.

logic saline solution. The cut-up material was thoroughly mixed with a small quantity of soil, obtained from a place on which no hogs had been kept for a number of years; in most cases the soil was obtained from the back yard of a dwelling in the southwest section of Washington, D.C., an area on which no swine had ever been kept so far as could be determined.

A number of earthworms were then placed in a glass jar, or other suitable glass container, to which the contaminated soil had been added; the container was covered loosely with a glass dish. After the earthworms had remained in the small quantity of soil for from 1 to 3 days, during which time at least part of the soil passed through the alimentary canal of the annelids as shown by the presence of typical casts in the containers, the earthworms were removed to another glass container to which a considerable quantity of uncontaminated soil had been added; the top layer of soil was covered with several layers of moist filter paper, water being added to the filter paper from time to time to keep the soil damp. Under these conditions the earthworms burrowed in the soil during the day, much in the same way as they do in nature. During the day some earthworms were usually found between the layers of the filter paper.

When the earthworms were kept under these conditions at room temperature they usually remained alive for weeks, although they frequently decreased in size, presumably as the result of starvation, since no food of any kind other than that present in the soil was available to them. In occasional cultures a heavy mortality of earthworms was observed, particularly during the first few days. In such cases the dead earthworms were removed and examined for larvae, and the live worms were transferred to new containers with fresh soil. When deaths occurred transfers were made daily, and this procedure usually enabled the writers to carry along cultures of earthworms uninterruptedly for several weeks.

The anterior portion of the alimentary canal, including the pharynx, esophagus, crop, gizzard, and anterior portion of the intestine of the earthworms, was examined for larvae; in most cases the hearts, the dorsal blood vessel, and occasionally the ventral blood vessel, were also examined for evidence of infestation. For the purpose of determining the morphological status of the larvae, they were removed from their locations in the intermediate host, with the aid of dissecting needles, and examined microscopically.

In feeding experiments with pigs and dogs these animals were force-fed intact earthworms which had been exposed to infestation for a period adequate for the development of the larvae to the infective stage. The guinea pigs and other animals used were force-fed the carefully dissected anterior portion of the alimentary canal and adjoining blood vessels, including the hearts of infested earthworms.

Earthworms used in feeding experiments were collected in places to which swine had not had access for a number of years, so far as could be determined. Practically all the earthworms used in experiments were collected at the Bureau of Animal Industry Experiment Station at Bethesda, Md. Several earthworms from each lot intended for experimental infection were examined for larvae; the results were negative in nearly all cases. Occasionally individual

earthworms were found to harbor slight infestations; more than three larvae were seldom found, but such lots were not used in experiments involving development of the larvae in the intermediate host.

All pigs used in transmission experiments were from litters which had been farrowed and subsequently reared on concrete floors; the pigs were kept under careful supervision so as to preclude extraneous infestations with lungworms. During the course of this investigation, as well as of investigations with reference to other swine parasites, conducted during the past few years by the writers and others in the Zoological Division, no evidence of lungworm infestation was ever found in any pig reared under conditions described above.

Fecal examinations to determine the presence of eggs in the droppings of infested swine were made by the salt-flotation technic.

Post-mortem examinations of mammalian hosts were made as follows: The mesenteric lymph glands were torn apart with the aid of dissecting needles and small portions were examined in succession in press preparations. Portions of the liver and lungs were examined in a similar way. When larvae were not found in these organs, the latter were cut up with a pair of fine scissors and examined with the aid of the Baermann apparatus. When larvae were present, they readily came down the stem of the funnel and could easily be collected for study.

Field studies on the prevalence and abundance of earthworms in hog pastures, in lots, and elsewhere, were made in points in Maryland, Georgia, and to some extent in North Carolina. Earthworms were obtained from fields and other places by digging in the soil. The specimens collected were placed in glass containers with sufficient soil and examined in the laboratory when convenient.

PROTOCOLS ON EXPERIMENTAL INFECTIONS OF EARTHWORMS

INFECTIONS WITH METASTRONGYLUS ELONGATUS

EXPERIMENT 1

On August 7, 1929, five earthworms, species undetermined, were placed in soil containing eggs and larvae of *Metastrongylus elongatus*. Individual earthworms were dissected to determine degree and extent of infestation, as shown in the following results:

Earthworm 1, August 13. Five first-stage larvae, 525μ long by 20μ wide recovered from hearts, no examination made of other organs. Esophagus of typical larva about 130μ long; excretory pore about 80μ and genital primordium about 130μ from posterior end; tail 40μ long.

Earthworm 2, August 15. A number of larvae recovered from hearts. Each of these worms had a loose sheath, indicating a molt.

Earthworm 3, August 16. Larvae of three types recovered from this earthworm, namely, (1) first-stage larvae which had not yet molted; (2) larvae which had two sheaths and had, therefore, molted twice; and (3) larvae which had already cast off sheath of first molt but retained that of second molt.

Earthworm 4, August 23. Numerous third-stage larvae recovered from hearts, dorsal blood vessel, and parietal blood vessels.

Earthworm 5, September 5. Numerous larvae, 625 by actual count, recovered from same location as noted in connection with earthworm 4.

EXPERIMENT 2

On August 26, 1929, several earthworms, species undetermined, were placed in soil containing eggs and larvae of *Metastrongylus*

elongatus. Dissection of one of these intermediate hosts was made with the following results:

Earthworm 6, August 27. Only one larva in intestinal contents; other organs and tissues examined with negative results.

EXPERIMENT 3

On August 27, 1929, several earthworms, species undetermined, were placed in soil infected with *Metastrongylus elongatus*. One earthworm showed the following infestation:

Earthworm 7, September 6. Hearts contained number of larvae with two sheaths and some larvae which had already cast off first sheath but still retained second sheath. One larva, with two sheaths, discarded first sheath while under observation.

EXPERIMENT 4

On October 13, 1930, a number of earthworms, *Helodrilus foetidus*, were exposed to infection with *Metastrongylus elongatus*. Post-mortem examinations were made of 12 of these with the following results:

Earthworms 8 and 9, October 15. No. 8 contained three first-stage larvae in wall of posterior portion of esophagus; a few larvae in blood vessels. No. 9 contained 12 larvae in wall of posterior portion of esophagus; no larvae in blood vessels. Larvae recovered from earthworm 9 were 275μ to 310μ long by 15μ wide; esophagus 100μ to 110μ long.

Earthworm 10, October 18. Only one larva in wall of posterior portion of esophagus; no larvae in blood vessels.

Earthworm 11, October 20. Twenty-eight first-stage larvae in wall of esophagus, in posterior portion for the most part; in the four larvae examined, no evidence of molt; no larvae in blood vessels.

Earthworm 12, October 22. Fifty larvae in wall of esophagus; a few larvae in anterior portion; majority in posterior portion; 1 larva recovered from hearts.

Earthworm 13, October 23. Ten larvae in wall of posterior portion of esophagus and 9 larvae in various locations in wall of anterior portion of esophagus; no larvae in blood vessels. Of larvae examined, none showed evidence of molting.

Earthworm 14, October 24. Ten first-stage larvae in wall of posterior portion of esophagus; no larvae in blood vessels.

Earthworm 15, October 27. Twenty-three larvae in wall of posterior portion of esophagus; several had undergone first molt but retained sheath. Two of these second-stage larvae had the following measurements: Length, 580μ and 600μ; width, 28μ; length of esophagus, 125μ and 130μ; distance of genital primordium from anterior end, 320μ and 325μ; length of tail, 35μ.

Earthworm 16, October 28. About 25 second-stage larvae in wall of posterior portion of esophagus; no larvae in blood vessels.

Earthworm 17, October 29. More than 30 larvae in wall of posterior portion of esophagus; 2 larvae in hearts, these undergoing second molt, since each had two sheaths.

Earthworm 18, November 4. One earthworm contained about 150 larvae in wall of esophagus, larvae being located in posterior portion for the most part; 3 larvae in first pair of hearts, 1 in second pair, 1 in third pair, and 4 in fourth pair.

Earthworm 19, November 17. About 200 third-stage larvae found; about a dozen larvae in each of the five pairs of hearts, remaining larvae being located in wall of posterior portion of esophagus.

EXPERIMENT 5

On October 13, 1930, a number of earthworms, *Helodrilus caliginosus* var. *trapezoides*, were placed in soil containing eggs and larvae

of *Metastrongylus elongatus*. Eight of the earthworms were examined at various intervals with the following results:

Earthworm 20, October 15. Six first-stage larvae in wall of posterior part of esophagus; no larvae in blood vessels.

Earthworm 21, October 18. Five larvae in wall of esophagus, 2 being in anterior portion and 3 in posterior portion. Larvae had following measurements: Length, 275μ to 300μ; width, 15μ; length of esophagus, 100μ to 110μ.

Earthworm 22, October 20. Forty-two first-stage larvae in wall of posterior portion of esophagus; no larvae in blood vessels.

Earthworm 23, October 23. Six larvae in wall of posterior portion of esophagus; none in blood vessels.

Earthworm 24, October 29. Thirty-five larvae in wall of posterior portion of esophagus; of 10 larvae examined, 4 had 2 sheaths each and 6 had only 1 sheath each. One larva in one of first pair of hearts.

Earthworm 25, November 4. About 30 larvae in first pair of hearts; several of these appeared to be disintegrated.

Earthworms 26 and 27, November 17. No. 26 contained 3 larvae in wall of posterior part of esophagus; no. 27 contained more than 75 larvae in same location and 2 larvae in first pair of hearts.

EXPERIMENT 6

On November 21, 1930, several earthworms, *Helodrilus foetidus*, were placed in soil containing eggs and larvae of *Metastrongylus elongatus*. Examinations of four earthworms were made at various intervals after exposure, with the following results:

Earthworm 28, November 29. About 100 larvae in wall of posterior portion of esophagus; no larvae in blood vessels.

Earthworm 29, December 5. More than 200 larvae in posterior portion of wall of esophagus; larvae numerous in all hearts. Most larvae examined were in second stage; a few larvae had two sheaths each.

Earthworm 30, December 13. A total of 320 larvae in hearts and in wall of esophagus.

Earthworm 31, December 26. About 500 larvae in wall of posterior part of esophagus; hearts contained numerous larvae; all larvae in third stage.

EXPERIMENT 7

On April 8, 1931, several earthworms, species undetermined, were placed in soil containing eggs and larvae of *Metastrongylus elongatus*. Four earthworms were dissected, with results as follows:

Earthworm 32, April 15. Numerous first-stage larvae in wall of posterior part of esophagus; no larvae in blood vessels. Larvae had following measurements: Length 375μ to 400μ; width, 15μ; length of esophagus, 125μ to 133μ; distance of nerve ring, excretory pore, and genital primordium from anterior end, 70μ, 76μ, and 260μ, respectively.

Earthworm 33, April 19. First-stage larvae recovered from wall of esophagus; a few larvae undergoing first molt.

Earthworm 34, April 22. Larvae in second stage recovered from posterior wall of esophagus. One of these larvae had the following measurements: Length, 600μ; width, 26μ; length of esophagus, 180μ; distance of nerve ring and excretory pore from anterior end, 76μ and 85μ, respectively; length of tail, 50μ.

Earthworm 35, April 26. Many third-stage larvae found.

EXPERIMENT 8

On August 26, 1931, several earthworms, species undetermined, were exposed to infection with *Metastrongylus elongatus*. One earthworm was dissected with the following results:

Earthworm 36, September 22. Larvae abundant in wall of gizzard, some in wall of crop, many in wall of intestine but only in region immediately posterior to crop, large numbers in hearts.

INFECTIONS WITH CHOEROSTRONGYLUS PUDENDOTECTUS

EXPERIMENT 1

On August 22, 1929, one earthworm, species undetermined, was placed in soil containing eggs and larvae of *Choerostrongylus pudendotectus*. This earthworm was examined with the following results:

Earthworm 37, September 3. Two larvae in one heart, one of these larvae being 372μ long.

EXPERIMENT 2

On August 28, 1929, several earthworms, species undetermined, were placed in soil containing eggs and larvae of *Choerostrongylus pudendotectus*. One of these earthworms was examined with the following results:

Earthworm 38, December 7. Contained numerous larvae in wall of esophagus; none in blood vessels.

EXPERIMENT 3

On October 13, 1930, a number of earthworms, *Helodrilus caliginosus* var. *trapezoides*, were placed in soil containing eggs and larvae of *Choerostrongylus pudendotectus*. Five of these earthworms were examined with the following results:

Earthworm 39, October 15. Only one larva in posterior portion of esophageal wall; none in blood vessels.

Earthworm 40, October 18. Fourteen larvae in wall of posterior portion of esophagus; none in blood vessels.

Earthworm 41, October 25. Fifteen larvae in wall of posterior portion of esophagus; none in blood vessels.

Earthworm 42, November 1. About 65 third-stage larvae in wall of posterior portion of esophagus, 4 larvae in first pair of hearts, 2 in each of second pair, and 1 in one of third pair of hearts.

Earthworm 43, November 15. Six larvae in first pair of hearts, 3 in one of second pair, and 3 in wall of posterior portion of esophagus.

EXPERIMENT 4

On October 13, 1930, a number of earthworms, *Helodrilus foetidus*, were exposed to soil containing eggs and larvae of *Choerostrongylus pudendotectus*. Seven of these earthworms were examined with the following results:

Earthworm 44, October 15. About a dozen first-stage larvae in wall of posterior portion of esophagus; these larvae 285μ to 310μ long by 15μ wide; no larvae in blood vessels.

Earthworm 45, October 18. Two first-stage larvae in wall of posterior portion of esophagus; no larvae in blood vessels.

Earthworm 46, October 21. Forty-three first-stage larvae in wall of posterior portion of esophagus and a few in wall of anterior portion; none in blood vessels.

Earthworm 47, October 23. Fifteen first-stage larvae scattered in various portions of esophageal wall; none in blood vessels.

Earthworm 48, October 29. Twenty-four larvae, mostly in wall of posterior portion of esophagus, several larvae undergoing first molt; none in circulatory system.

Earthworn 49, November 4. One hundred and twenty-five third-stage larvae, mostly in posterior portion of esophagus; 4 larvae in first pair of hearts and 2 in one of fourth pair of hearts.

Earthworm 50, November 17. About 78 third-stage larvae in wall of posterior portion of esophagus; 4 larvae in first pair of hearts, 18 in second pair, 9 in third pair, 1 in one of fourth pair, and 1 in one of fifth pair of hearts; about 36 larvae in dorsal blood vessel.

EXPERIMENT 5

On November 21, 1930, several earthworms, *Helodrilus caliginosus* var. *trapezoides*, were placed in soil containing eggs and larvae of *Choerostrongylus pudendotectus*. Four of the earthworms were examined with the following results:

Earthworm 51, November 21. About 100 first-stage larvae in wall of posterior portion of esophagus; none in hearts.

Earthworm 52, December 5. About 150 larvae in posterior portion of wall of esophagus; 1 larva in dorsal blood vessel; a few larvae undergoing second molt.

Earthworm 53, December 13. About 130 larvae in posterior portion of wall of esophagus; none in blood vessels; most larvae in third stage.

Earthworm 54, December 26. About 150 third-stage larvae scattered in esophageal wall, mostly in posterior portion; numerous larvae in one heart and several in each of remaining hearts.

EXPERIMENT 6

On April 8, 1931, several earthworms, species undetermined, were placed in soil containing eggs and larvae of *Choerostrongylus pudendotectus*. Four of the earthworms were examined with results as follows:

Earthworm 55, April 15. Many larvae in posterior portion of wall of esophagus; none in hearts; all in first stage. Larvae had following measurements: Length, about 360μ to 385μ; width, 15μ; length of esophagus, 123μ to 130μ; distance of genital primordium from anterior end, 240μ to 275μ.

Earthworm 56, April 19. Several larvae in posterior portion of wall of esophagus; one first-stage larva 390μ long by 15μ wide; esophagus 130μ long; genital primordium 210μ from anterior end.

Earthworm 57, April 22. Most larvae in posterior portion of wall of esophagus; larvae undergoing second molt. Measurements as follows: Length, 580μ to 610μ; width, 26μ; length of esophagus, 140μ to 160μ; distance of nerve ring, excretory pore, and genital primordium from anterior end, 72μ to 76μ, 79μ to 87μ, and 320μ to 355μ, respectively; length of tail, 45μ to 50μ.

Earthworm 58, April 26. Many larvae in esophagus and hearts; all in third stage.

INFECTIONS WITH METASTRONGYLUS SALMI

On February 21, 1933, 7 earthworms (6 of which were *Helodrilus caliginosus* var. *trapezoides* and 1 *Lumbricus terrestris*) were placed in soil containing eggs and larvae of *Metastrongylus salmi*. Later they were dissected to determine the degree of infestation, with results as follows:

Earthworm 59, February 22. A few first-stage larvae in wall of esophagus; two larvae were 275μ and 300μ long by 12μ wide.

Earthworm 60, March 1. Several first-stage larvae in wall of esophagus; one larva 380μ long by 18μ wide.

Earthworm 61, March 6. Several larvae in wall of esophagus, undergoing first molt. Three larvae were 500μ to 525μ long by 22μ to 26μ wide.

Earthworm 62, March 8. Four larvae from esophageal wall were undergoing second molt; larvae 550μ to 610μ long by 26μ to 28μ wide.

Earthworm 63, March 11. Several third-stage larvae and larvae undergoing second molt; two third-stage larvae 550μ long by 26μ wide.

Earthworm 64 (*Lumbricus terrestris*), March 13. A few third-stage larvae in esophageal wall.

Earthworm 65, March 23. Several third-stage larvae in posterior esophageal wall and in hearts; three third-stage larvae 550μ to 630μ long by 26μ wide.

INFECTIONS WITH UNDETERMINED SPECIES

On March 8, 1930, several earthworms, *Lumbricus terrestris*, were exposed to infection on soil to which eggs and larvae of undeter-

mined species of swine lungworms had been added about a week previously. One earthworm was examined with the following results:

Earthworm 66, March 20. Several infective larvae in hearts and in wall of esophagus adjacent to crop.

PROTOCOLS ON NATURAL INFESTATION OF EARTHWORMS WITH LARVAE OF SWINE LUNGWORMS

During the summer of 1929 the senior author made an investigation on farms in various parts of Maryland to determine the extent of natural infestation of earthworms with the larvae of swine lungworms. The results were as follows:

Farm 1. Of 10 earthworms, *Helodrilus caliginosus* var. *trapezoides*, from a hog pasture, 6 were negative and 4 positive; from 1 to 2 larvae found in individual earthworms.

Farm 2. Of 4 earthworms, *Helodrilus foetidus*, all were infested; they contained 25, 179, 80, and 30 larvae, respectively. Larvae in walls of esophagus, crop, gizzard, and intestine; comparatively few in hearts. Lot from which earthworms were collected had been vacant for several months, the pigs having been removed early that spring.

Farm 3. Earthworms, *Helodrilus foetidus* and *H. caliginosus* var. *trapezoides*, collected from old bare hog lot. Of 25 earthworms, 8 were negative and 17 positive; number of larvae in individual earthworms ranged from 1 to 25.

Farm 4. Of 10 earthworms, species undetermined, 9 were negative and 1 positive, the latter containing 2 larvae in hearts.

Farm 5. Of 5 specimens, *Helodrilus foetidus*, 2 were negative and 3 positive; one of latter contained about 50 larvae located in wall of esophagus and in hearts, another contained about 35 larvae in wall of esophagus, 3 in wall of crop and intestine, and none in hearts, and the third contained 1 degenerated larva.

Farm 6. Of 5 earthworms, species undetermined, collected near farm residence on area to which pigs had no access, all were negative.

Additional field studies on the occurrence of naturally infested earthworms were made by the senior author in southern Georgia in February 1931, in order to determine the prevalence of earthworms on temporary swine pastures as compared with their prevalence in pine woods and insanitary hog lots. These studies were made to determine also the extent and degree of infestation of earthworms with lungworm larvae in a part of Georgia which is a swine-growing area. The following results were obtained:

Farm 7. Of 8 earthworms, species undetermined, from pasture 1, all were negative; 2 earthworms from pasture 2 also negative. Of 7 earthworms from edge of pine woods, 3 were negative and 4 contained disintegrated larvae. Three sows, examined post mortem, were negative for lungworms. Earthworms found in lowest portion of pastures 1 and 2; pasture 1, which was more level than pasture 2, contained few earthworms. Except for the temporary pastures, no earthworms found in soil full of roots, but readily obtained near cow and horse manure. In one pasture with a hard soil, no earthworms were found.

Farm 8. Of 9 earthworms, species undetermined, 6 were negative and 3 contained from one to a dozen larvae.

Farm 9. Of 10 earthworms, *Helodrilus caliginosus* var. *trapezoides*, 7 were negative and 3 positive; the latter contained 2, 3, and 50 larvae, respectively, in wall of posterior part of esophagus and in hearts. Post-mortem records on pigs from this farm showed lungworm infestations, individual pigs harboring from 3 to 72 lungworms.

Farm 10. Of 8 earthworms, 7, species undetermined, were negative, and 1, *Helodrilus caliginosus* var. *trapezoides*, contained 2 larvae in hearts. Of 5 earthworms, species undertermined, from pine woods adjacent to temporary pastures, 3 were negative and 2 positive; the latter contained 2 and 3 larvae,

respectively, in wall of posterior part of esophagus. Four pigs, examined post mortem, contained 5 to 18 lungworms each.

Farm 11. Of 7 earthworms, *Helodrilus caliginosus* var. *trapezoides*, obtained along lane leading to horse barn, all were negative. Of 15 earthworms, species undetermined, from a temporary pasture, 14 were negative and 1 contained 1 larva in wall of esophagus; earthworms were obtained in fair numbers in lowest portion of pasture only. Of 7 earthworms from a woods pasture, 6 were negative and 1 contained 2 larvae, 1 in wall of esophagus and 1 in heart. Earthworms were obtained in lane near barns, on shaded side only, showing that such areas are possible sources of infection; these earthworms, however, were not examined. Post-mortem examination of 22 pigs showed 16 to be free from lungworms and 6 to be very slightly infested.

Farm 12. Of 7 earthworms, species undetermined, from pine woods, 5 were negative and 2 contained 500 and 2,000 larvae in wall of esophagus and in hearts. In temporary pasture which contained a hog pen, no earthworms were found in pasture, but they were present along fence of pen. Of 5 earthworms, *Helodrilus caliginosus* var. *trapezoides*, 4 were negative and 1 contained 10 larvae in wall of esophagus. Numerous earthworms were found in rich, damp soil at branch head of a stream in pine woods adjacent to pasture.

Farm 13. No earthworms in temporary pasture. Woods adjacent to pasture, lower than latter and accessible to pigs, contained numerous earthworms; soil in woods rich in humus, overgrown with grass, but fairly soft. Of 11 earthworms, species undetermined, from this soil, 5 were negative and the remaining 6 were infested, as follows: 3 contained 2, 2, and 3 larvae, respectively, in wall of esophagus; 1 contained 16 larvae in hearts and 2 in wall of esophagus; 1 contained about 100 larvae in wall of esophagus; and 1 contained about 150 larvae in location last mentioned.

Farm 14. Of 5 earthworms, species undetermined, from unfenced pasture adjacent to pine woods, 3 were negative and 2 contained 6 and 2 larvae, respectively, in wall of esophagus. Prior to examination, earthworms had been kept in refrigerator for 15 days; larvae active when examined.

Farm 15. Of 11 earthworms, species undetermined, from a small lot, uncultivated for about 10 years and containing rich soil covered with straw and cow and hog manure, 4 were negative and 7 positive; latter contained from 2 to 15 larvae in wall of esophagus, especially in posterior portion. Of 5 earthworms, species undetermined, from woods, 3 were negative and 2 positive; latter contained 2 and 9 larvae, respectively, in wall of esophagus. Earthworms kept in ice box 15 days before being examined; lungworm larvae recovered were active.

PROTOCOLS ON EXPERIMENTAL INFECTION OF VARIOUS ANIMALS WITH LUNGWORMS

PIGS

EXPERIMENT 1

On October 29, 1929, each of 6 pigs out of a litter of 8, farrowed October 18, 1929, was fed 10 earthworms which had been previously exposed to an infection with *Metastrongylus elongatus*. On November 8, 1929, each of the remaining 2 pigs, nos. 7 and 8, of this litter was fed the esophageal portions of 5 earthworms which had been exposed to a mixed infection of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*. The pigs were killed with the following results:

Pig 1, November 2. Only one larva in a mesenteric lymph gland of small intestine; exact identity of larva not determined.

Pig 2, November 5. Free from lungworms.

Pig 3, November 22. Contained 52 specimens of *Metastrongylus elongatus;* 2 largest females, 24 mm long by 296μ wide and 25 mm long by 312μ wide, respectively, contained several embryonated eggs in uteri; most of eggs observed were in morula stage; 1 female, 18 mm long and 280μ wide, contained eggs in morula stage. No larvae found in liver and lymph glands; lungs showed several hemorrhagic areas and slightly discolored pneumonic areas at tips.

Pigs 4 and 5, March 7 and 10, respectively. Free from lungworms.

Pig 6, March 14. Contained 24 fully grown lungworms; females contained embryonated eggs.

Pig 7, November 13. Lungs showed petechial hemorrhages; 6 females and 1 male worm isolated from these hemorrhages had the following measurements: Male—length, 800μ; width, 32μ. Females—length, 1.3 to 1.62 mm; width, 45μ to 53μ; length of esophagus, 210μ to 217μ; width of esophagus, 24μ to 26μ; distance of vulva from tip of tail, 57μ to 60μ; length of tail, 38μ to 41μ. Three larvae in process of degeneration found in lymph glands of pig; 1 of them was 620μ long by 30μ wide.

Pig 8, March 14. Contained several adults of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*.

EXPERIMENT 2

On January 3, 1930, one pig, farrowed October 18, 1929, was fed earthworms previously exposed to an infection with *Metastrongylus elongatus*. The animal was killed with the following results:

Pig 9, January 8. Lungs contained several petechial hemorrhages; one larva in each of three hemorrhagic areas examined; no larvae in lymph glands or liver. One larva from lungs was a fourth-stage female, 755μ long by 31μ wide; vulva 61μ from tip of tail.

EXPERIMENT 3

On April 22, 1930, two pigs, farrowed April 14, 1930, were fed earthworms infected with mixed cultures of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*. The animals were killed with the following results:

Pig 10, April 23. No larvae in intestinal contents or mesenteric lymph glands.

Pig 11, April 24. No larvae in intestinal contents or mesenteric lymph glands. Liver appeared normal; lungs showed few petechial hemorrhages, but no larvae were recovered.

EXPERIMENT 4

On May 12, 1930, two pigs, farrowed April 14, 1930, were fed earthworms infested with *Metastrongylus elongatus*. The animals were killed with the following results:

Pig 12, May 15. Twenty-seven larvae in mesenteric lymph glands adjacent to large intestine: larvae undergoing third molt; no larvae found in liver. Larvae from lymph glands were 530μ to 575μ long by 30μ wide; esophagus 140μ to 160μ long; tail 20μ to 50μ long.

Pig 13, May 20. Several larvae in washings of bronchi and bronchioles; all worms appeared to be in fifth stage. Males—2 to 3.4 mm long by 59μ to 76μ wide; esophagus 249μ to 280μ long. Females—3.5 to 4.2 mm long by 65μ to 78μ wide; esophagus 327μ long; vulva 69μ to 79μ from tip of tail; tail 46μ to 55μ long. Lungs of this pig showed several petechial hemorrhages.

EXPERIMENT 5

On June 9, 1930, one pig, farrowed April 14, 1930, was fed earthworms infested with *Metastrongylus elongatus*. This pig was killed with the following results:

Pig 14, June 12. Lungs showed many small hemorrhages containing larvae undergoing molt; several larvae in a mesenteric lymph gland adjacent to large intestine; no larvae in liver.

EXPERIMENT 6

On December 26 and 27, 1930, six pigs, farrowing date unknown, were fed earthworms infested with lungworm larvae; pigs 15, 16, 17, and 18 received earthworms infested with *Metastrongylus elongatus*, and pigs 19 and 20 received earthworms infested with *Choe-*

rostrongylus pudendotectus. Temperature readings taken on these pigs daily from December 26, 1930, to January 5, 1931, did not show any marked variation from the normal. Feces collected March 9, 1931, on pigs 16 and 18 were mixed with sand, and each sample was placed in a separate jar. Earthworms placed in these jars were killed March 22 and found to contain lungworm larvae in various stages of development in the esophageal wall. The pigs were killed on the dates given with the following results:

Pig 15, January 30, 1931. Lungs contained 610 adults of *Metastrongylus elongatus;* no worms or lesions noted in liver.

Pig 16, March 6. Lungs contained 326 adults of *Metastrongylus elongatus;* no lesions or worms noted in liver.

Pig. 17, March 6. No lungworms found.

Pig. 18, June 25. Lungs contained 136 adults of *Metastrongylus elongatus.*

Pig 19, July 1. No lungworms found.

Pig. 20, July 17. Lungs contained 1 adult female of *Choerostrongylus pudendotectus.*

EXPERIMENT 7

On May 9, 1931, each of two 3-month-old pigs were fed eight earthworms infested with larvae of *Choerostrongylus pudendotectus.* The pigs were killed with the following results:

Pig 21, May 12. Five larvae in the mesenteric lymph glands, 3 being fourth-stage males, 1, a female in process of shedding the two skins, and 1 a third-stage larva. Males—624μ to 680μ long by 32μ to 37μ wide; esophagus 180μ to 190μ long; nerve ring and excretory pore 72μ to 76μ and 87μ to 91μ, respectively, from anterior end. Female—750μ long by 347μ wide; esophagus 200μ long; nerve ring and excretory pore 76μ and 91μ, respectively, from anterior end; tail 41μ long. Third-stage larva—430μ long by 32μ wide; tail 41μ long. A young fifth-stage female found in a mesenteric lymph gland was 960μ long by 32μ wide; esophagus 220μ long; nerve ring and excretory pore 100μ and 106μ, respectively, from anterior end; vulva 72μ from posterior end, the ovary extending 197μ from vulva. No hemorrhagic areas found in lungs of pig.

Pig 22, May 14. Lungs showed several petechial hemorrhages; one young adult male was recovered from one of these areas; bursa of male apparent, but rays incompletely formed; worm 1.11 mm long by 45μ wide; nerve ring and excretory pore 91μ and 121μ, respectively, from anterior end; spicules somewhat slender and about 418μ long.

GUINEA PIGS

Several guinea pigs weighing between 350 and 375 g were fed infective lungworm larvae with negative results. Young guinea pigs weighing 250 g or less were infested experimentally with results as follows:

EXPERIMENT 1

On December 27, 1932, each of five guinea pigs was fed a number of infective larvae of *Metastrongylus elongatus* isolated from earthworms. Post-mortem results on these animals are as follows:

Guinea pig 1, fed 100 larvae, killed January 26, 1933. No worms recovered; several grayish spots on surface of lungs; other organs normal.

Guinea pig 2, fed 250 larvae, killed January 26. No worms recovered; upper lobe of right lung showed red hepatization with a few scattered hemorrhages; surface of liver contained several white spots.

Guinea pig 3, fed 500 larvae, killed January 20. Two males and sixteen females of *Metastrongylus elongatus* recovered from lungs, which were completely consolidated; females contained unsegmented eggs in uteri.

Guinea pig 4, fed 750 larvae, killed January 26. No lungworms recovered; posterior tips of lungs showed red hepatization; other organs normal.

Guinea pig 5, fed 1,000 larvae, killed January 26. No worms recovered; upper lobes of lungs showed red hepatization; few hemorrhagic areas in lower portions of main lobes; other organs normal.

EXPERIMENT 2

On July 28, 1932, each of two guinea pigs was fed the esophagi and hearts of six earthworms previously exposed to infection with the eggs and larvae of *Choerostrongylus pudendotectus* and *Metastrongylus elongatus*. These animals were killed on the dates shown, with the following results:

Guinea pig 6, killed July 29, exactly 24 hours after feeding of infective lungworm larvae. Mesenteric lymph glands of large intestine contained 45 third-stage larvae. Two of these larvae 525μ and 550μ long by 25μ and 26μ wide; esophagus 175μ and 210μ long; genital primordium 255μ and 286μ from anterior end; tail 30μ long. Liver contained 1 third-stage larva 440μ long by 26μ wide; esophagus 175μ long; genital primordium 255μ from anterior end; tail 175μ long.

Guinea pig 7, killed July 30, 48 hours after feeding of infective larvae. Yielded 56 third-stage larvae from mesenteric lymph glands, especially those near cecum; 1 larva from liver; larvae from lymph glands wider than those obtained from guinea pig 6. Two larvae each 525μ long by 30μ wide; esophagus 210μ long; genital primordium 280μ from anterior end; tail 30μ long. In 1 larva genital primordium 35μ long, considerably larger than those in larvae obtained from guinea pig 6.

EXPERIMENT 3

On July 30, 1932, each of three guinea pigs was fed the esophagi and hearts of three earthworms previously exposed to infection with eggs and larvae of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*. Post-mortem examinations of these animals showed the following results:

Guinea pig 8, killed August 2, approximately 72 hours after experimental infestation. Mesenteric lymph glands yielded 62 larvae; most larvae surrounded by two sheaths and 1 larva had already cast off both sheaths. Two sheathed female larvae 660μ and 675μ long by 30μ wide; esophagus 200μ and 225μ long; vulva 15μ from anus; tail 41μ and 45μ long. Larva which had already cast off both sheaths was 700μ long by 30μ wide; esophagus 190μ long; vulva 22μ from anus; tail 45μ long. Male larvae from lymph gland, with two sheaths, readily recognizable by inflated posterior portion destined to form bursa. Lungs had number of petechiae; of 3 female larvae recovered from lungs, 2 had two sheaths each and 1 had already cast off sheaths. Sheathed larvae 740μ long by 30μ wide; esophagus 190μ long; vulva 22μ from anus; tail 45μ long. No larvae in blood of heart, in spleen, pancreas, or liver.

Guinea pig 9, died August 10. On August 9 this animal showed labored breathing, suggestive of pneumonia. On post-mortem examination 35 young fifth-stage lungworms recovered from lungs; others present in these organs not removed. Some females recognizable as *Choerostrongylus pudendotectus;* others, as males and females of *Metastrongylus elongatus;* one of the latter contained two shelled eggs in uteri. Measurements of young fifth-stage worms were as follows: *M. elongatus:* One male—length, 4.8 mm; width, 98μ; width of esophagus, 349μ; distance of nerve ring and excretory pore from anterior end, 152μ and 228μ, respectively; length of spicules, approximately 1.68 mm. Two females—length, 5.4 and 8.1 mm; width, 83μ and 98μ; length of esophagus, 380μ and 402μ; distance of nerve ring from anterior end, 144μ and 197μ; distance of vulva from tip of tail, 91μ and 129μ; length of tail, 68μ and 83μ.

Choerostrongylus pudendotectus: One female—length, 4 mm; width, 106μ; length of esophagus, 319μ; distance of nerve ring from anterior end, 182μ; distance of vulva from tip of tail, 182μ.

Guinea pig 10, died August 9, after 4:30 p.m.; examined August 10. On August 8 showed respiratory difficulty suggestive of pneumonia; on post-mortem examination, lungs presented mottled appearance and contained petechiae; microscopic section of lungs showed congestion of blood vessels in air sacs and blood in sacs; lungworms in air sacs. No larvae in mesenteric lymph

glands or liver; 42 lungworms recovered from lungs; undetermined number not recovered. Female lungworms well advanced in development, ovaries being fairly well developed; male worms had well-formed bursa supported by rays. Largest specimens (1 male and 1 female) had following measurements: Female—length, 7 mm; width, 93μ; length of esophagus, 436μ; distance of nerve ring and excretory pore from anterior end, 200μ and 280μ, respectively; length of tail, 78μ. Male—length, 2.5 mm; width, 62μ; length of esophagus, 275μ; length of spicules, 450μ.

EXPERIMENT 4

On August 10, 1932, each of two guinea pigs was fed the esophagi and hearts of three earthworms previously exposed to infection with *Choerostrongylus pudendotectus* and *Metastrongylus elongatus*. These animals were killed with the following results:

Guinea pig 11, killed September 7. At beginning of experiment animal weighed 240 g; prior to death, 227 g. Posterior portion and borders of lungs showed thorough consolidation; 2 well-developed and 13 smaller specimens of female *Metastrongylus elongatus* in bronchus of right lung. All worms contained shelled eggs in uteri, but none were embryonated; 1 egg, not yet embryonated, in mucus of bronchus. Worms from 20 to 27 mm long by 202μ to 296μ wide; esophagus 530μ to 577μ long; nerve ring 300μ to 311μ from anterior extremity; vulva 114μ to 129μ from tip of tail; tail 83μ to 114μ long. No specimens of *Choerostrongylus pudendotectus* were found.

Guinea pig 12, killed September 10. At beginning of experiment animal weighed 250 g; shortly before death animal was emaciated and weighed only 200 g. Prior to death, respiration was labored, fur rough, and animal barely moved about. On post-mortem examination, upper lobes of lungs and tips of lower lobes were consolidated; bronchi contained pus in which 25 females and 1 male, all adult forms of *Metastrongylus elongatus*, were found. The females contained shelled eggs, not embryonated, in uteri; eggs, 53μ to 57μ long by 38μ to 41μ wide, appeared abnormal, contents being shrunken. Measurements of worms were as follows: Two largest females—length, 27 and 29 mm; width, 296μ and 327μ; length of esophagus, 546μ and 624μ; length of tail, 78μ and 91μ. One male—length, 14mm; width, 202μ; length of esophagus, 405μ; length of spicules, about 3.34 mm.

DOGS

On August 5, 1932, two dogs, about 1 month old, were fed 6 and 8 earthworms, respectively, which had been previously exposed to an infection with *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*. Post-mortem examination of these animals gave the following results:

Dog 1, fed eight earthworms, died August 24. Five lungworms, *Metastrongylus elongatus*, recovered from bronchi; 42 lungworms, 1 being *Choerostrongylus pudendotectus* and the others *M. elongatus*, recovered from bronchioles; all lungworms in fifth stage. One female *M. elongatus* contained eggs in uteri, the eggs being in the one- and two-cell stages; specimen of *C. pudendotectus*, a female, had well-developed ovaries and genital organs but contained no eggs in uteri. Interior portion of right lobe of lungs completely consolidated, remaining portions of this lobe showing scattered hemorrhages and triangular areas of consolidation along margins. General appearance of lungs suggestive of broncho-pneumonia; bronchi and bronchioles contained pus.

Dog 2, fed six earthworms, killed September 6. A week before animal was killed it coughed considerably. Fourteen lungworms, of which 13 were *Metastrongylus elongatus* and 1 *Choerostrongylus pudendotectus*, were found in bronchi and bronchioles. All lungworms recovered were females 8 to 17 mm long by 109μ to 187μ wide; not all lungworms were recovered, however, since fragments were seen in press preparations of lung after removal of parasites already mentioned. Posterior lateral borders of lungs showed consolidation; appearance of lungs suggestive of broncho-pneumonia; bronchi filled with pus in which 2 lungworm eggs were found; these eggs, as well as those in uteri of females, not embryonated.

OTHER MAMMALIAN HOSTS

On November 1, 1929, one earthworm heavily infested with larvae of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus* was fed to a white rat. Post-mortem examination of this experimental host on November 5 resulted in the discovery in one of the mesenteric lymph glands of only 1 larva, a male, with the 2 sheaths of the final molt.

In view of the fact that *Metastrongylus elongatus* has been reported on a few occasions as a parasite of man, an experiment was carried out to determine the susceptibility of a primate to an infection with this lungworm. On March 3, 1931, a monkey, *Macaca lasiotis*, was fed several hundred infective larvae of *Metastrongylus elongatus*, obtained from an earthworm dissected the previous day. The monkey was killed on March 25 and examined for evidence of lungworm infestation, with negative results.

On August 10, 1932, an earthworm which had been infected experimentally with swine lungworms and which harbored infective larvae was fed to a cat. Post-mortem examination of this experimental animal on September 8 failed to disclose the presence of lungworms.

HATCHING OF EGGS AND LONGEVITY OF ARTIFICIALLY HATCHED LARVAE

The infective embryonated eggs, obtained from gravid females, were of two types, namely, thin shelled and thick shelled (fig. 1). Thin-shelled eggs are seldom deposited, since observations made by the writers have failed to disclose the presence of thin-shelled eggs in the manure of infested swine. The thin-shelled embryonated egg is one in which shell formation is as yet incomplete. The thickening of the shell is accompanied by a contraction, and this produces an uneven, somewhat mammillated surface which bears a resemblance to the mammillated albuminous layer of *Ascaris* eggs.

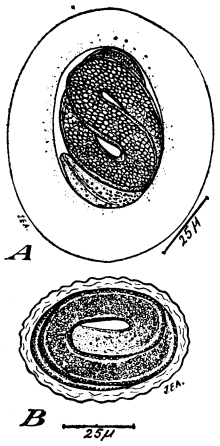

FIGURE 1.—*A*, Thin-shelled egg of *Metastrongylus elongatus*; *B*, thick-shelled egg of *M. elongatus*.

The supposition made by some investigators, that hatching of the egg may occur regularly while it is on its way out of the host animal, was not confirmed in the course of this investigation. Examinations of scrapings from the tracheae and bronchi of infested swine failed to reveal larvae, but nearly always showed the presence of thick-shelled eggs. Furthermore, in the numerous examinations of swine manure by the writers with the aid of the Baermann apparatus and in other ways, lungworm larvae were found in only two instances, and in very small numbers. On the other hand, examination of manure from infested swine by the salt flotation technic revealed the characteristic lungworm eggs with thick shells, an observation first made by the junior author, and later confirmed by the senior author.

Under normal conditions the eggs of lungworms parasitic in swine are eliminated with the feces of the infested animals and do not

hatch ordinarily until they are taken into the bodies of susceptible earthworms, while the latter are feeding on swine manure or on soil and debris contaminated with such manure. Experimentally, earthworms become readily infested with the larvae which issue from the as yet incompletely formed (thin-shelled) eggs. These larvae, as noted by the Hobmaiers (*5*), the writers, and various other investigators, are long-lived in water, and, as observed by the writers, appear to be unaffected by putrefactive changes which might take place in this medium. As noted by the senior author, the artificially hatched larvae (fig. 2) usually become quiescent in water and, after a few days' maintenance in that medium, they show little sign of activity unless stimulated by heat, mechanical pressure, or in some other way. Since the behavior of larvae is the same in water and soil, the larvae, therefore, are not adapted to a free-living existence in soil, and hatching under normal conditions is probably accidental. The thick-shelled eggs also remain viable in water media but do not hatch even after prolonged maintenance in water at room temperature.

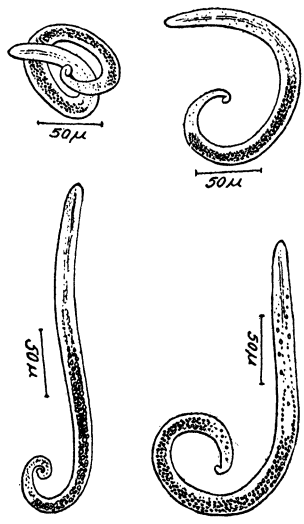

FIGURE 2.—Artificially hatched lungworm larvae (species undetermined) from water cultures maintained at room temperature. (Original figures prepared by B. H. Ransom.)

Among the unpublished notes left by the late B. H. Ransom, of the Bureau of Animal Industry, are data which have a bearing on the longevity of artificially hatched swine lungworm larvae. Two cultures of larvae, one in water and the other in a mixture of sterile feces and charcoal, were made on February 5 (year not stated) and kept at room temperature. Examinations of these cultures were made nearly every day. As late as March 21, more than 5 weeks after the cultures were started, a live larva was found in the charcoal culture. Five larvae were found in the water culture as late as March 31. On April 3 this culture was dry; water was added to it, but the larvae were all dead when examined on April 5. The Hobmaiers (*5*) maintained larvae of *Metastrongylus elongatus* in a moist medium for 3 months; the larvae were alive at the end of this period.

The further development of embryos present in infective eggs or of artificially hatched larvae which remain viable can take place only in suitable earthworm hosts, so far as is known.

SPECIES OF EARTHWORMS IN WHICH SWINE LUNGWORM LARVAE DEVELOPED

An undetermined number of species of earthworms were found naturally infested with species of swine lungworms, and several species of earthworms were experimentally infected with the larvae of these parasites. However, only 4 species of earthworms involved in this investigation were authoritatively determined by Frank

Smith, formerly of the University of Illinois; 3 species were determined specifically, and 1 was determined generically only.

The specifically determined species were (1) *Helodrilus foetidus* (synonyms *Allolobophora foetida* and *Eisenia foetida*), an earthworm of common occurrence in manure, compost heaps, and in soil rich in manure and other decaying matter; (2) *H. caliginosus* var. *trapezoides* (synonym *A. caliginosa trapezoides*), which occurs in practically the same locations as *H. foetidus;* and (3) *Lumbricus terrestris*, a species not encountered by the writers on animal pastures but readily obtained on park lawns, especially during and after heavy rains. These three species belong to the family Lumbricidae.

A species of *Diplocardia* (family Megascolecidae), collected by H. B. Raffensperger, in charge of the Bureau's zoological field station at Moultrie, Ga., and experimentally infected by him with undetermined species of swine lungworms, could not be determined specifically owing to the fact that the specimens of this species available to the writers had not yet attained a state of reproductive activity. Specimens of these experimentally infected earthworms were examined by the writers, and second-stage lungworm larvae were isolated from them. Of the undetermined earthworms used in this investigation, one species, a very active form moving in quick, serpentine fashion, was obtained from marshes in South Carolina, and other species, possibly distinct from those already referred to, were obtained on temporary pastures in Georgia and elsewhere. Every species of earthworm subjected by the writers to an experimental infection with any of the three species of swine lungworms proved to be a suitable host.

Von Schuckmann and Zunker (*12*) reported that *Eisenia foetida* (synonym of *Helodrilus foetidus*) was a suitable intermediate host for *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*. These writers stated also that although larvae of swine lungworms developed in *Allolobophora caliginosa* (synonym of *H. caliginosus*) and in *Lumbricus terrestris*, many were destroyed in these intermediate hosts. They noted further the failure of swine lungworms to develop in one species of earthworm, *Bismatus tenuis*. In *L. rubellus* Von Schuckmann and Zunker found a natural infestation with infective lungworm larvae.

MIGRATION OF SWINE LUNGWORMS IN THE INTERMEDIATE HOST

The three species of swine lungworms showed no significant difference in the rate of their development or in the details of their migrations in earthworms. The life cycle is treated, therefore, as a unit for the most part.

On being ingested by suitable earthworms with soil, manure, or in other ways, the eggs hatch and the larvae, once they are free in the lumen of the alimentary canal of the intermediate host, penetrate its wall. It is possible that hatching is aided by mechanical pressure of particles of soil against the shells. Hatching probably takes place very soon after the eggs are taken in by earthworms, presumably in the upper part of the alimentary canal; however, this point has not been determined. When earthworms are exposed to soil to which the artificially hatched larvae or thick-shelled eggs or both have been added, infestation takes place readily.

The larvae accumulate in the wall of the esophagus of earthworms, especially in that of the posterior portion, and penetrate occasionally the wall of the crop, rarely that of the gizzard, and occasionally that of the intestine. The larvae probably penetrate the wall of the alimentary canal soon after hatching, but whether they penetrate the wall of the esophagus or reach their locations in the esophagus by upward migration from the wall of the intestine, gizzard, or crop has not been determined. Whether the accumulation of larvae in the wall of the lower portion of the esophagus is governed by chemotactic influences or can be explained in some other way still remains to be determined. It is of interest to note, however, that the wall of the esophagus of earthworms is well supplied with blood vessels, and, as noted by the writers, the region where lungworm larvae tend to accumulate appears to be highly vascular. On the other hand, it must not be overlooked that, though the wall of the pharynx of earthworms is also highly vascular, no larvae were encountered in this location.

The path of migration of the larvae from the wall of the esophagus, crop, and intestine to the blood vessels has not been definitely traced. It has been observed, however, as shown in the protocols, that the occurrence of larvae in the dorsal blood vessel and their accumulation in the hearts usually take place subsequent to their accumulation in the wall of the alimentary canal; in the latter location the larvae can and do develop to the infective stage, as shown in figure 3. Heavy infections with larvae in the wall of the esophagus, together with the absence of larvae in blood vessels, have been noted in naturally infested earthworms as well as in those experimentally infected. Migration of larvae from the wall of the intestines to the blood vessels appears to be an accidental occurrence rather than an essential part of the migrations of the larvae in the intermediate host. In *Lumbricus*, the dorsal blood vessel lies on the alimentary canal, adherent to the intestine in its entire length, except in the region of the hearts; since this blood vessel receives blood from other vessels, the transfer of lungworm larvae from the wall of the intestine to the lumen of the dorsal blood vessel can result either from the migration of larvae in the blood stream or from their active penetration into that blood vessel. Since the blood in the dorsal vessel flows forward, it would naturally carry along, sooner or later, larvae which might enter it. From the dorsal blood vessel the blood enters the ventral vessel through the hearts, and this naturally explains the accumulation of the larvae in the latter locations (fig. 4). The larvae are probably arrested in the hearts by the valves and are thus prevented from entering the ventral blood vessel, in which the blood flows in a backward direction. This explains, probably, the rather rare occurrence of larvae in the ventral blood vessel. Figure 5, a diagrammatic sketch of an experimentally infected earthworm, dissected to show the distribution of larvae in various locations, gives a graphic view of the actual findings in the case of one experimental infection involving *Metastrongylus elongatus*. The actual number of larvae found and their location and distribution are reproduced in this illustration.

Von Schuckmann and Zunker (*12*) expressed the view that lungworm larvae do not linger in the wall of the alimentary canal but

migrate to the blood vessels almost immediately after penetrating the wall of the esophagus, a view which is out of harmony with the data presented in this bulletin. Figure 3 shows a very heavy infesta-

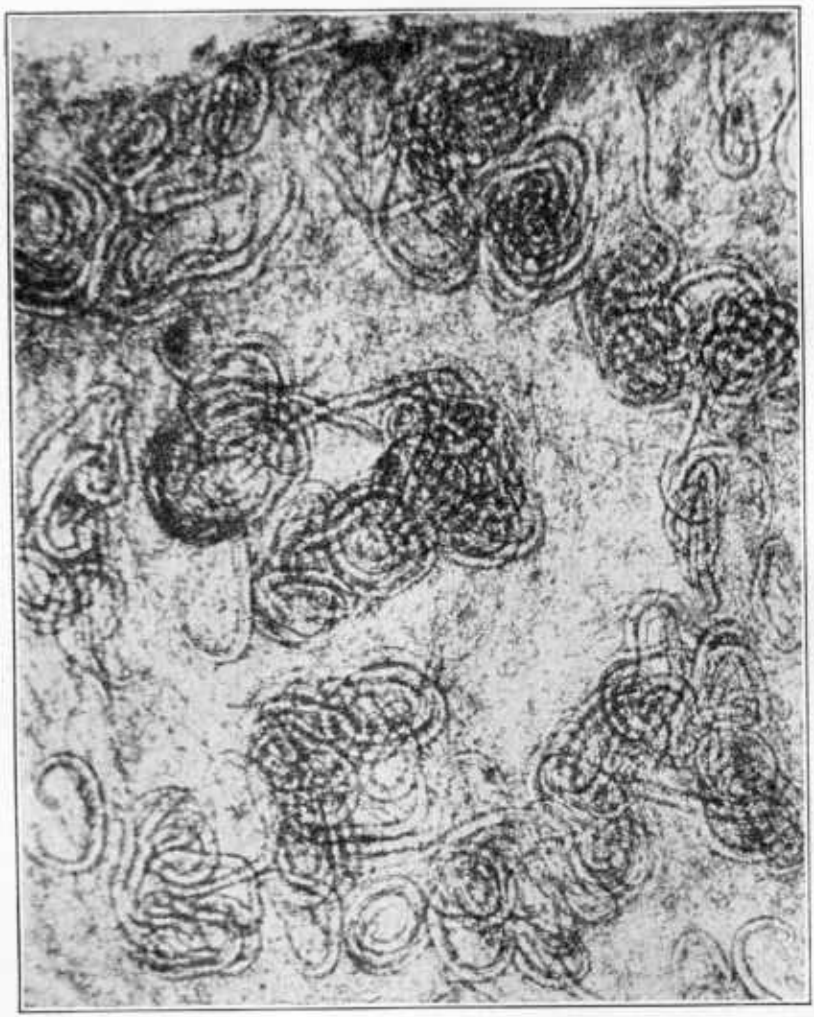

FIGURE 3.—Portion of the posterior half of the esophagus of an earthworm (press preparation) showing lungworm larvae embedded in esophageal wall. The earthworm was found naturally infested with undetermined species of swine lungworms. ×75.

tion of lungworm larvae in the wall of the esophagus of an earthworm naturally infested with these parasites; the larvae in question were in the infective (third) stage.

MOLTING OF LARVAE IN THE INTERMEDIATE HOST

Growth and development of the larvae, accompanied by two molts, take place in the wall of the intestine and can take place also in the

blood vessels of the intermediate host. As shown in the protocols and elsewhere in this bulletin, first-stage larvae, when hatched artificially, are less than 400μ long; these larvae attain a length up to 540μ before molting.

The two molts occur in rather rapid succession. In one series of experiments with *Metastrongylus elongatus*, carried out in August, the first molt was observed as early as the ninth day after exposure of earthworms to eggs and larvae of this parasite (fig. 6, *A*), and the

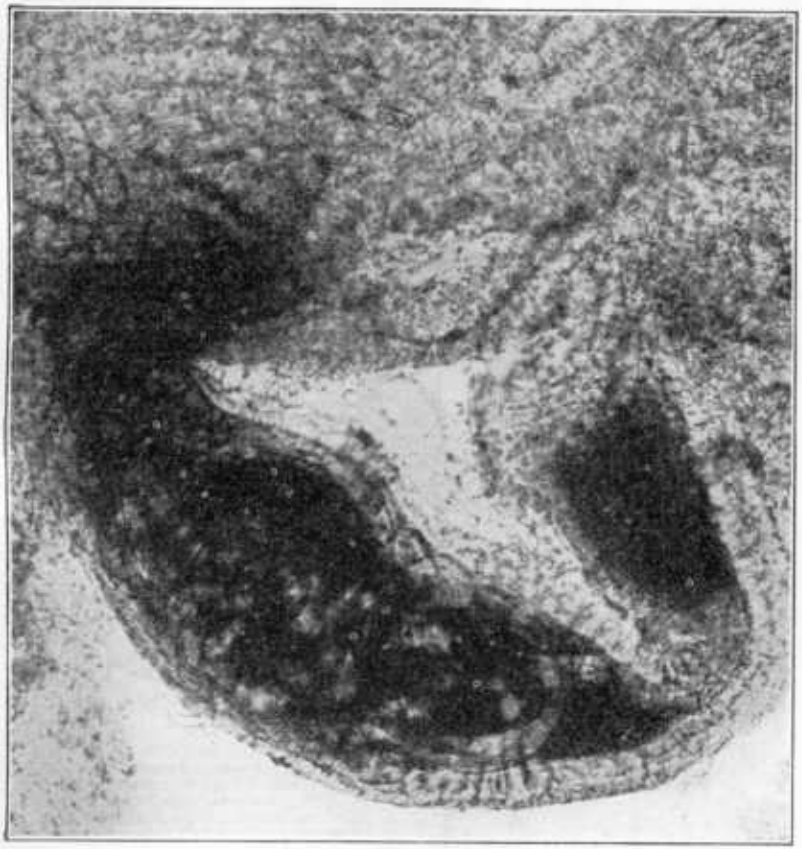

FIGURE 4.—One of the hearts of an earthworm with lungworm larvae, *Metastrongylus elongatus*, inside. Infection produced experimentally. ×225.

second molt was observed the next day (fig. 6, *B*). In another series of experiments, carried out in October, larvae obtained from an earthworm 12 days after exposure of the intermediate host to infection, had not yet undergone the first molt; however, 3 days later, or 15 days after exposure, one earthworm contained larvae with the loose sheath of the first molt. In another earthworm involved in this experiment, no larvae were found in the second molt until the seventeenth day after it had been exposed to infection. In another series of experiments, also involving *Metastrongylus elon-*

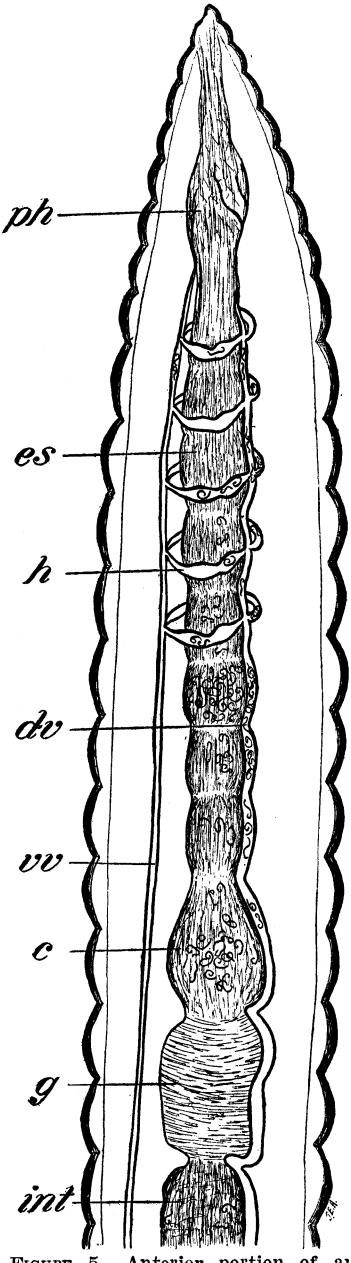

FIGURE 5.—Anterior portion of an earthworm showing locations of lungworm larvae, *Metastrongylus elongatus*, in wall of intestine and in circulatory system: *c*, Crop; *dv*, dorsal blood vessel; *es*, esophagus; *g*, gizzard; *h*, heart; *int*, intestine; *ph*, pharynx; *vv*, ventral blood vessel.

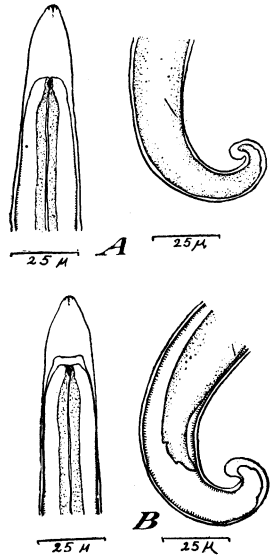

FIGURE 6.—*A*, Sections of *Metastrongylus elongatus* larva in first molt: Left, anterior portion; right, posterior portion. *B*, Sections of *M. elongatus* larva in second molt: Left, anterior portion; right, posterior portion.

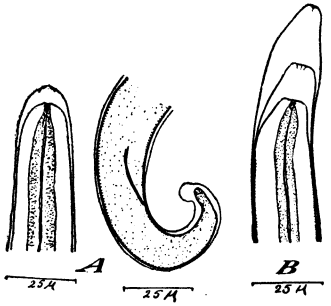

FIGURE 7.—*A*, Sections of *Choerostrongylus pudendotectus* larva in first molt: Left, anterior portion; right, posterior portion. *B*, Anterior portion of *C. pudendotectus* larva in second molt.

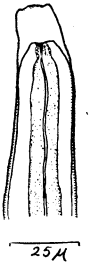

FIGURE 8.—Anterior portion of infective (third-stage) larva of *Metastrongylus elongatus* with the sheath of only the second molt present.

gatus, carried out in October, second- and third-stage larvae were found 12 days after the earthworms had been exposed to infection. In one series of experiments with *Metastrongylus elongatus*, carried out during November and December, second- and third-stage larvae were found in earthworms 14 days after these intermediate hosts had been exposed to infection.

In an experiment involving *Choerostrongylus pudendotectus*, second-stage larvae still in the first molt were encountered 17 days after the earthworm had been exposed to infection (fig. 7, *A*). In another experiment with this species, carried out in November and December, third-stage larvae were encountered 15 days after the earthworms had been exposed to infection (fig. 7, *B*). In one experiment with *Choerostrongylus pudendotectus* carried out in April, third-stage larvae were found 14 days after the intermediate host had been exposed to infection.

In one experimental infection of earthworms with *Metastrongylus salmi*, carried out during the summer of 1933, first-stage larvae were found in the wall of the posterior portion of the esophagus about 30 hours after the earthworms had been exposed to infection; on the thirteenth day after exposure larvae in the first molt were found in the wall of the esophagus; 2 days later larvae with 2 sheaths were found in the same location in another earthworm; 18 days after exposure, 1 earthworm contained larvae enveloped in 1 sheath, that of the second molt, the larvae being located in the wall of the esophagus and in the hearts.

It appears, from these data, that the development of swine lungworms in earthworms varies to some extent with the temperature of the surrounding medium, development being more rapid in warm weather than in cool weather in some cases, and that the second molt commences within a day, or a little later, after the first molt has become evident.

As with other molting nematode larvae, the separation of the cuticle from the body is seen, at first, in the head and tail regions (figs. 6 and 7). The sheath of the first molt is apparently not cast off before that of the second molt has become separated from the body of the larva, these points being clearly brought out in figures 6*B*, 7*B*, and 10*B*. Larvae with the sheaths of the molts still adhering to them were commonly encountered in the course of this study, as shown in the protocols. However, either during or after the completion of the second molt, the sheath of the first molt is discarded, and the infective third-stage larva is now surrounded by one sheath, that of the second molt (fig. 8). The differentiation of second- and third-stage larvae, when only one sheath is present, is not difficult, since the anterior end of the first sheath is conical, whereas that of the second sheath is truncated and characterized by liplike structures.

During the process of growth and metamorphosis, accompanied by molting, the larvae continue to increase in size, those of *Metastrongylus elongatus* attaining a length of 685μ when infective, those of *Choerostrongylus pudendotectus*, so far as observed, attaining a length of 625μ when infective. *M. salmi*, of which only one experimental infection was made, attained a length of 630μ as an infective larva.

DESCRIPTIONS OF DEVELOPMENTAL STAGES OF LUNGWORMS IN THE INTERMEDIATE HOST

METASTRONGYLUS ELONGATUS

EGGS

Eggs eliminated with the manure of infested swine and ingested by earthworms are 45μ to 57μ long by 38μ to 41μ wide (fig. 1, *B*), elliptical in shape, thick shelled, and contain an embryo enclosed in a vitelline membrane; the surface of the shell is roughened. Thin-shelled embryonated eggs (fig. 1, *A*) present in the uteri are also capable of infecting earthworms; these eggs are 87μ to 100μ long by 72μ to 91μ wide. The shells are very permeable to water and swell considerably in water cultures before the larvae hatch.

FIRST-STAGE LARVAE

Artificially hatched first-stage larvae (fig. 9, *A*) are from 275μ to 305μ long by 12μ wide. The anterior end is blunt and somewhat narrowed; the posterior end, also somewhat narrowed, is characteristically coiled and bent ventrad. Except for narrowing at the ends, the body of a typical larva is more or less uniform in diameter. The esophagus is from 114μ to 117μ long, with a double swelling; the excretory pore posterior to first swelling is approximately 75μ from the anterior end. The intestine is dark and granular in contrast to the distinctly lighter esophagus; the rectum is well marked off from the intestine.

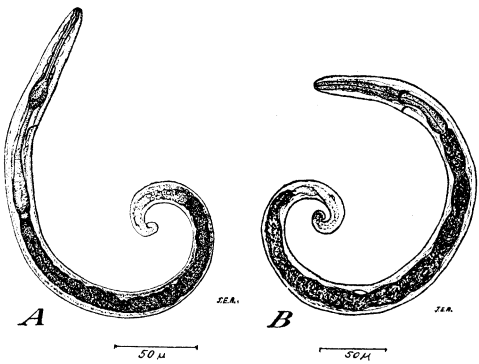

FIGURE 9.—*A*, Artificially hatched larva of *Metastrongylus elongatus* from water culture maintained at room temperature, showing morphological details. *B*, First-stage larva of *M. elongatus* from one of the hearts of an earthworm 6 days after experimental infection.

The first-stage larvae in the wall of esophagus usually, and in hearts occasionally, grow and attain a length of from about 500μ to 540μ. Figure 9, *B* shows a first-stage larva obtained from one of the hearts of an earthworm 6 days after exposure of the intermediate host to eggs and larvae. This larva, approximately 500μ long, has a club-shaped esophagus about 115μ long; the nerve ring is posterior to the middle of the esophagus, and the excretory pore is slightly posterior to the nerve ring. The intestine is dark and granular, in contrast to the light esophagus; the rectum is well marked off from the intestine; the tail is short and still characteristically coiled and bent ventrad.

SECOND-STAGE LARVAE

In the second stage (fig. 10, *A*) the larva is surrounded by a sheath which is conical at its anterior end. Larva, exclusive of sheath, is about 550μ to 650μ long by 26μ to 28μ wide, more or less truncated at the anterior end, and attenuated toward the posterior end. The esophagus is more or less club-shaped, 125μ to 160μ long;

with the anterior swelling less distinct than in the first stage. The nerve ring is about 75μ from the anterior end; excretory pore slightly posterior to nerve ring; intestine of more or less uniform width. The genital primordium is about 320μ to 350μ from the anterior end, its location being posterior to the middle of intestine, dividing latter into two parts having an approximate ratio of 4 to 3. The rectum is about 35μ long; the tail, slightly notched, from 35μ to 60μ long.

THIRD-STAGE LARVAE

The early third-stage larva (fig. 10, *B*) has the sheath of the first and second molts; infective third-stage larva (fig. 10, *C*) is surrounded by the sheath of the second molt, truncated at anterior end and showing two characteristic indentations, giving the appearance of lips. Larvae of one series, obtained from an earthworm about 1

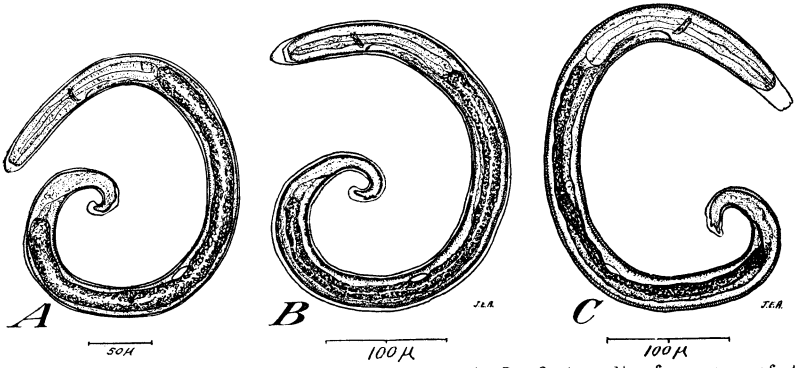

FIGURE 10.—Larva of *Metastrongylus elongatus*: *A*, In first molt; from one of the hearts of an earthworm 8 days after experimental infection. *B*, In second molt; from one of the hearts of an earthworm 9 days after experimental infection. *C*, Infective (third stage); from one of the hearts of an earthworm 9 days after experimental infection.

month after experimental infection, had the following measurements: Length, 650μ to 685μ; width, 26μ; length of club-shaped esophagus, 150μ to 170μ; distance of nerve ring and excretory pore from anterior end, 75μ to 85μ and 83μ to 91μ, respectively; length of tail, 40μ to 60μ, which tapers gradually and usually possesses two deep notches near its end. In this series, the excretory pore of the larva led into long excretory glands extending to posterior portion of larva; the genital primordium was posterior to middle of intestine. The measurements of another series of infective third-stage larvae are given in table 1.

TABLE 1.—*Principal measurements of 10 third-stage (infective) larvae of Metastrongylus elongatus*

Item	Measurements, in microns, of larva no.									
	1	2	3	4	5	6	7	8	9	10
Length of body	625	660	630	685	625	640	625	685	625	665
Maximum width of body	26	26	26	26	26	26	26	26	26	26
Length of esophagus	170	170	170	170	150	170	170	180	170	170
Distance from excretory pore to anterior end	83	91	83	91	91	91	91	91	88	91
Distance from genital primordium to anterior end	360	375	365	365	350	365	360	390	355	360
Length of tail	50	50	40	50	50	60	50	40	50	45

CHOEROSTRONGYLUS PUDENDOTECTUS

EGGS

Thick-shelled eggs are 60μ to 64μ long by 43μ to 45μ wide, similar in shape and general appearance to those of *Metastrongylus elongatus*. Thin-shelled eggs from uterus are 91μ to 106μ long by 80μ to 99μ wide, also similar in shape to those of *M. elongatus*.

FIRST-STAGE LARVAE

Artificially hatched larvae are 325μ to 375μ long by 15μ wide (fig. 11, *A*), similar to those of *Metastrongylus elongatus*. The esophagus is 125μ to 130μ long; the nerve ring slightly posterior to commencement of last third of esophagus. Growth takes place as

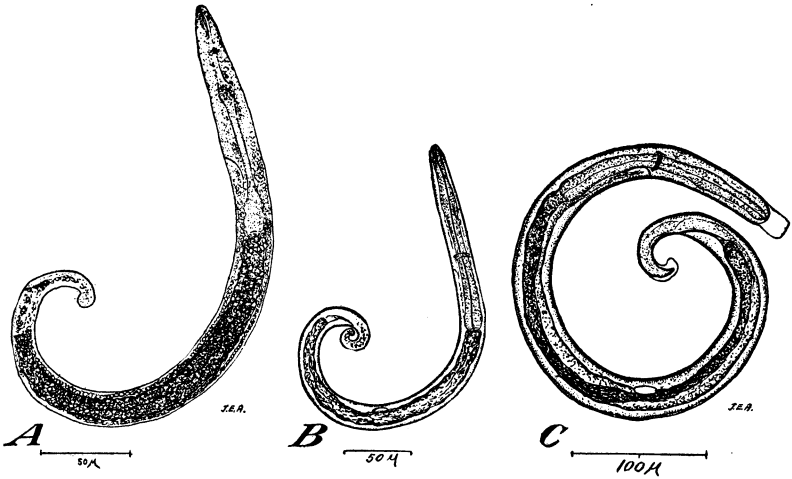

FIGURE 11.—Larva of *Choerostrongylus pudendotectus*: *A*, Just after hatching from thin-shelled egg; *B*, first stage, from esophageal wall of earthworm 7 days after experimental infection; *C*, infective (third stage) 15 days after experimental infection of earthworm.

in *M. elongatus*, the larvae (fig. 11, *B*) attaining a length of about 525μ at the beginning of the first molt.

SECOND-STAGE LARVAE

The second-stage larvae are similar in measurements to those of *Metastrongylus elongatus*, being 590μ to 630μ long by 26μ to 28μ wide; esophagus 136μ to 160μ long; nerve ring and excretory pore 72μ to 76μ and 79μ to 87μ, respectively, from anterior end; genital primordium 340μ to 355μ from anterior end; tail 45μ to 50μ long.

THIRD-STAGE LARVAE

The third-stage larvae (fig. 11, *C*) are similar to those of *Metastronglus elongatus*. One series of larvae had the following measurements: Length, 600μ to 625μ; width, 26μ; length of esophagus, 160μ to 190μ; distance of nerve ring and excretory pore from anterior end, 75μ to 82μ and 84μ to 87μ, respectively; distance of genital primordium from anterior end, 350μ to 390μ; length of tail, 55μ to 62μ, usually with two indistinct notches at the posterior end. Typical variations in morphology of tail are shown in figure 12. Another series of measurements involving 10 larvae is given in table 2.

TABLE 2.—*Principal measurements of 10 third-stage (infective) larvae of Choerostrongylus pudendotectus*

Item	Measurements, in microns, of larva no.									
	1	2	3	4	5	6	7	8	9	10
Length of body	630	585	610	600	615	565	580	600	570	625
Maximum width of body	26	26	26	26	26	26	26	26	26	26
Length of esophagus	170	170	165	170	180	155	160	175	160	165
Distance from excretory pore to anterior end	91	87	85	85	87	91	83	83	86	91
Distance from genital primordium to anterior end	365	335	350	350	355	315	345	300	320	357
Length of tail	60	55	55	55	60	55	55	60	55	62

METASTRONGYLUS SALMI

EGGS

The thick-shelled eggs are 43μ to 57μ long by 38μ to 41μ wide, similar in shape and general appearance to those of the other two species. The thin-shelled eggs are 72μ to 86μ long by 53μ to 60μ wide.

FIRST-STAGE LARVAE

Only a few artificially hatched first-stage larvae were measured; these were 275μ to 295μ long by 12μ wide. These larvae are similar to those of the other two species and, like the latter, increase in size as they develop in earthworms, attaining a length of 500μ to 525μ and a width of 22μ to 26μ at the beginning of the first molt. At this time the esophagus is from 120μ to 130μ long; nerve ring and excretory pore, 60μ to 70μ and 72μ to 78μ, respectively, from anterior end; genital primordium, 280μ to 285μ from anterior end; tail, 38μ to 45μ long.

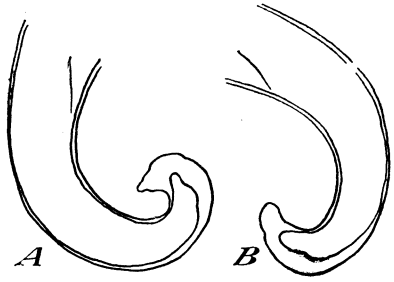

FIGURE 12.—Tails of two infective larvae of *Choerostrongylus pudendotectus* showing variations in structure: *A*, Tail with two indentations; *B*, tail with one indentation.

SECOND-STAGE LARVAE

The second-stage larvae, like those of the other two species, differ but slightly from the fully grown first-stage larvae. At the beginning of the second molt the larvae are 550μ to 610μ long by 26μ to 28μ wide; esophagus, 145μ to 160μ long; nerve ring and excretory pore, 68μ to 72μ and 78μ to 80μ, respectively, from anterior end; genital primordium, 290μ to 330μ from anterior end; tail, 48μ to 50μ long.

THIRD-STAGE LARVAE

The infective larvae are from 550μ to 630μ long by 26μ wide; esophagus, 150μ to 175μ long; nerve ring and excretory pore, 64μ to 72μ and 72μ to 80μ, respectively, from anterior end; genital primordium, 300μ to 345μ from anterior end; tail, 40μ to 50μ long, resembling in shape that of *Choerostrongylus pudendotectus*. .

NATURAL INFESTATIONS OF EARTHWORMS WITH LUNGWORM LARVAE AND DISTRIBUTION OF EARTHWORMS

As shown in the protocols, individual earthworms, naturally infested, have been found to harbor in some instances infestations as heavy as or heavier than those produced as a result of experimental exposure to infective material. Of the naturally infested earthworms which were dissected in the course of this investigation, only two species, *Helodrilus foetidus* and *H. caliginosus* var. *trapezoides*, were definitely identified; other species may have been involved. As already noted, Von Schuckmann and Zunker (*12*) observed natural infestations in *Lumbricus rubellus*.

As in the case of experimentally infected earthworms, the larvae in natural infestations, as observed by the writers, were present, for the most part, in the wall of the upper portion of the alimentary canal, especially in that of the posterior half of the esophagus; larvae were found in the hearts also in some cases. In a few instances larvae were found in a state of disintegration. The assignment of the latter to one of the species of swine lungworms appears to be justified, however, in view of their location either in the wall of the alimentary canal or in the hearts. It is worth noting in this connection, that Cori (*3*) described a nematode from the ventral blood vessel of *Lumbricus terrestris*. According to Cori, the larval worms in question were found exclusively in the ventral blood vessel throughout its entire length, a location in which swine lungworm larvae were rarely encountered. Aside from the difference in location, the larvae from the ventral blood vessel of *L. terrestris* were from 3 to 4 mm long by 100μ wide, and attenuated at each end; the tail was 160μ long and terminated in a knoblike tip. On the basis of this description, the possibility that these larvae were developmental stages of any of the species of swine lungworms can be definitely excluded. Cori regarded these larval worms as being the immature stages of a spirurid, *Spiroptera turdi* Molin.

Another nematode from the ventral blood vessel of earthworms was described by Friedlaender (*4*) under the name *Lumbricicola vasorum*. This form was studied in section only. Whether it represents a species distinct from that described by Cori, or whether the two are identical, cannot be determined from available information. Other nematodes which occur in earthworms can be readily differentiated from the larval forms discussed in this bulletin on the basis of morphology and location within the body of the oligochaete host.

Incidental observations on the abundance and distribution of earthworms on hog pastures and elsewhere showed that these annelids, at least the two identified species which were found to be naturally infested, were rare or practically absent from fields which had been under frequent cultivation, that they were not common in fields covered with carpet grass or other thick grass, and that they were practically absent from loose, dry, sandy soils. When earthworms were encountered in cultivated fields, they were usually found in the lower parts of pastures, especially along the fences.

Earthworms were found in abundance in uncultivated lots containing humus, in pine woods containing a rich and somewhat loose soil, in and about manure from various animals, especially that from

horses, cows, and pigs. Although a moist soil was found to be a favorite location for earthworms, a very wet or marshy soil appeared to be a rather unfavorable habitat for these annelids.

MIGRATION OF METASTRONGYLUS ELONGATUS AND CHOEROSTRONGYLUS PUDENDOTECTUS IN THE DEFINITIVE HOST

In view of the comparative scarcity of *Metastrongylus salmi* in swine, this species was not involved in experiments on the migration or development of lungworms in mammalian hosts. As noted in connection with the development and migration of lungworms in the intermediate host, no differences were detected in these respects in the species *M. elongatus* and *Choerostrongylus pudendotectus* in the definitive host, and the topic is therefore treated, except as otherwise noted, as a unit.

Following the ingestion by swine, or other suitable hosts, of earthworms harboring infective lungworm larvae, these larvae, freed from the intermediate host tissue by the process of digestion, evidently penetrate the wall of the intestine and on reaching the lymph spaces, follow the course of the lymph stream. This inference is justified in view of the accumulation of larvae in the mesenteric lymph glands, especially in those of the large intestine, and more particularly in the lymph glands near the cecum. This observation is in agreement with the observations of the Hobmaiers, (*5*, *6*) and of Von Schuckmann and Zunker (*12*). Although larvae were not encountered in the liver of pigs, observations in harmony with those of the Hobmaiers (*5*) and of Von Schuckmann and Zunker, they were encountered in two instances in the liver of guinea pigs, only a single larva being found in each case. Evidently the liver is only exceptionally invaded by the larvae.

The larvae encountered in the mesenteric lymph glands 1 or more days after experimental infection, probably succeed in extricating themselves from this location and ultimately reach the venous system by way of the thoracic duct. From the venous system the larvae naturally get to the lungs by way of the right side of the heart and the pulmonary arteries. Whether the larvae encountered in the liver of the guinea pig reached this organ by entering the venules of the intestine or those of the lymph glands cannot be stated on the basis of available information. In either case, the larvae could reach the liver by way of the portal vein. Whether the larvae could extricate themselves from the liver through the capillary circulation and enter the hepatic veins has not been determined in view of their scarcity in the liver. The Hobmaiers (*6*) expressed the view that swine lungworm larvae, because of their size, could not get through the capillary network of the liver.

The larvae that reached the lungs presumably bored through the walls of the capillaries and entered the alveolar spaces. Microscopic sections of the lungs of an experimentally infected guinea pig showed congestion of the blood vessels of the air sacs and the presence of blood and larvae within the alveoli, facts that fit in with the above assumption. From the alveolar spaces the larvae, as they continue to grow, would naturally have to migrate to the bronchioles, and in small experimental animals, such as guinea pigs,

they would have to wander up the bronchi. In swine, lungworms are located, for the most part, in the bronchioles, especially in those of the posterior border of the lungs. They also occur in the bronchi and occasionally in the trachea. The protocols on the development of lungworms in guinea pigs show that the young lungworms were recovered from the bronchi.

MOLTING OF LARVAE IN THE DEFINITIVE HOST

In guinea pigs as well as in pigs the two final molts take place in rapid succession. In guinea pigs killed 24 and 48 hours, respectively, after experimental infection, only third-stage larvae, advanced in development beyond that of the infective stage encountered in earthworms, were found. In guinea pigs killed 72 hours after experimental infection, larvae with two sheaths, those of the third and fourth molts, as well as larvae which had already discarded these sheaths, were discovered in the lymph glands and in the lungs. This shows that the fourth molt may take place in guinea pigs before the sheath of the third molt has been discarded. It shows further that larvae arrested in the mesenteric lymph glands of these animals undergo the last two molts in these locations. Probably the shedding of the two sheaths which, no doubt, hinder to some extent the movements of the larvae, enable the latter to extricate themselves from the lymph glands and reach their preferred location in the lungs.

As shown in the protocols, experimental infections of pigs with *Metastrongylus elongatus* and *Choerostrongylus pudendotectus*, respectively, yielded results similar to those obtained in guinea pigs. The larvae underwent 2 molts, which took place in the lymph glands or in the lungs. Third-stage larvae advanced in development beyond that of the infective stage, larvae in the third molt and those in the fourth molt with the sheaths of the final 2 molts still present, were encountered in pigs 3 days after experimental infection. These findings, as already noted elsewhere in this bulletin, are not in harmony with the observations of the Hobmaiers and of Von Schuckmann and Zunker, who noted only 2 molts in the course of the development of lungworms, 1 molt in the intermediate host and the other in the definitive host. According to their observations, lungworms pass through 3 stages and 2 molts, whereas the data presented in this bulletin show that swine lungworms, in common with other nematodes, pass through 5 stages and undergo 4 molts.

The development of lungworms after the two final molts involves further growth and differentiation. Although pigs killed and examined 3 days after experimental infection contained molting larvae for the most part, pigs killed 1 or 2 days later contained larvae which had already discarded their sheaths and were in the fifth stage. Molting larvae were usually less than 700μ long; 4-day-old worms in the fifth stage were from 750μ to somewhat over a millimeter long; 8-day-old worms were from 2 to 4.2 mm long, the smaller worms being males. A female of *Metastrongylus elongatus* contained what appeared to be fully embryonated eggs 24 days after experimental infection of a pig. Although these worms were not fully grown,

the presence of embryonated eggs in the uteri showed that the worms had already attained fertile maturity. The Hobmaiers (*5*) observed the presence of fully embryonated eggs in swine lungworms 30 days after experimental infection of pigs and before the female worms had attained full growth.

DESCRIPTIONS OF EARLY DEVELOPMENTAL STAGES OF LUNGWORMS IN THE DEFINITIVE HOST

METASTRONGYLUS ELONGATUS

FOURTH-STAGE LARVAE

Fourth-stage larvae cannot be differentiated easily on morphological grounds from young fifth-stage worms. Some larvae recovered from the mesenteric lymph glands of pigs 3 days after infection were undergoing the third molt (fig. 13) and were, therefore, early fourth-stage larvae. These larvae had the following morphological features: Body relatively stout, 530μ to 575μ long by 30μ wide; anterior end broadly rounded, posterior end rather attenuated and terminating in a knoblike process; esophagus club-shaped, 140μ to 160μ long; nerve ring and excretory pore 65μ and 70μ, respectively, from anterior end; tail 20μ to 50μ long, the short-tailed forms probably being males. Sex differentiation becomes more evident, however, in older fourth-stage larvae. The older male larvae of this stage have distended tails, indicating the beginning of the formation of the bursa; female larvae possess the primordium of the future vulva and have slender tails. One advanced fourth-stage female larva recovered from the lungs of a pig 5 days after experimental infection was 755μ long by 31μ wide; the primordium of the vulva was 51μ from the posterior end.

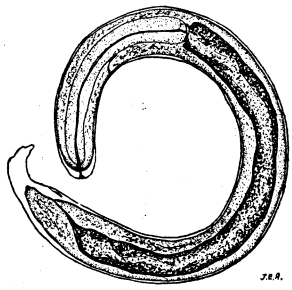

FIGURE 13.—Larva of *Metastrongylus elongatus* in third molt. From mesenteric lymph gland of pig 3 days after experimental infection.

FIFTH-STAGE WORMS

As already stated, larvae of *Metastrongylus elongatus*, in common with those of *Choerostrongylus pudendotectus*, develop rather rapidly in the final host, since fairly well-formed fifth-stage worms were recovered as early as 8 days after experimental infection. A description of these worms follows: Male—length, 2 to 3.4 mm; width, 59μ to 76μ; body slender, slightly tapering anteriorly and of same width for most of the length; esophagus (fig. 14) club-shaped, 249μ to 280μ in length; bursa (fig. 15) well formed, but its rays not so well defined as in fully developed worm. Female—length, 3.5 to 4.2 mm; width, 65μ to 78μ; body slender, slightly tapering anteriorly (fig. 16, *A*) and more so posteriorly (fig. 16, *B*); esophagus club-shaped, 327μ in length; vulva opening to the outside, 23μ from anal opening and 69μ to 76μ from posterior end; tail digitiform, 46μ to 53μ in length.

CHOEROSTRONGYLUS PUDENDOTECTUS

FOURTH-STAGE LARVAE

In view of the fact that in the larvae studied the fourth molt was already in evidence before the sheath of the third molt had been discarded, it was practically impossible to separate the fourth from

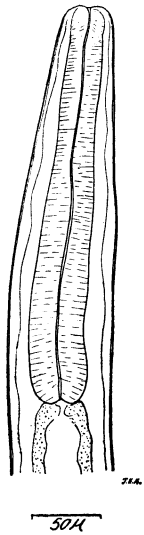

FIGURE 14.—Anterior portion of young male *Metastrongylus elongatus* from one of the bronchi of a pig 8 days after experimental infection.

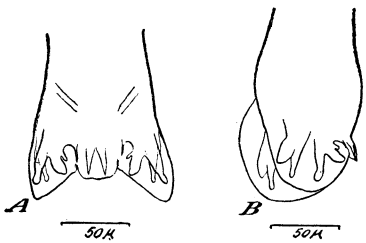

FIGURE 15.—Bursa of male *Metastrongylus elongatus* recovered from one of the bronchi of a pig 8 days after experimental infection: *A*, Bursa spread out; *B*, bursa in lateral view.

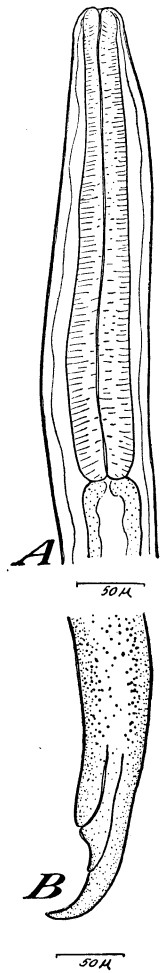

FIGURE 16.—Portions of female *Metastrongylus elongatus* recovered from one of the bronchi of a pig 8 days after experimental infection: *A*, Anterior portion; *B*, posterior portion.

the young fifth stage on morphological grounds. The following description is that of fourth-stage larvae at the beginning of the last molt (fig. 17, *A*), the larvae in question being surrounded by two sheaths. Males—length, 624μ to 628μ; maximum width, 32μ to 34μ; body slender and of same width for most of length; anterior end rounded, posterior portion, destined to form bursa, distended; esopha-

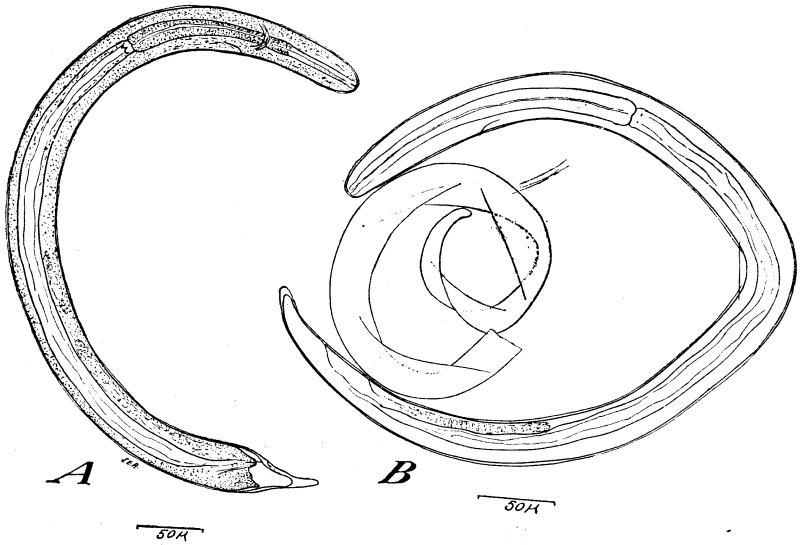

FIGURE 17.—*A*, Fourth-stage male larva of *Choerostrongylus pudendotectus* showing beginning of fourth molt. *B*, Female larva of *C. pudendotectus* with sheaths of two final molts; one sheath was discarded while larva was under observation. Both larvae from mesenteric lymph gland of pig 3 days after experimental infection.

gus club-shaped, 180μ to 190μ in length; nerve ring and excretory pore 72μ to 76μ and 87μ to 91μ, respectively, from anterior end; tail distended posteriorly and showing several indistinct lines and folds, indicating the future bursal lobes and rays; vas deferens already connected to rectum. Female—body slender, as in male, and of same width for most of length (fig. 17, *B*); one fourth-stage female with fourth molt in progress, 750μ in length and 34μ in maximum width; anterior portion slightly tapering and posterior portion somewhat slender and digitiform; esophagus 200μ in length and club-shaped; nerve ring and excretory pore, 76μ and 91μ, respectively, from anterior end; vulva apparent late in fourth stage but not opening to outside until final larval cuticle has been shed; vulva about 31μ anterior to anal opening, and 72μ from posterior end; gonoduct and ovary at this stage not clearly differentiated; tail digitiform, 41μ in length. The posterior portion of a female with the sheaths of the two final molts is shown in figure 18; the sheath of the third molt shows the anal opening of the fourth-stage larva but does not show an opening of the reproductive system.

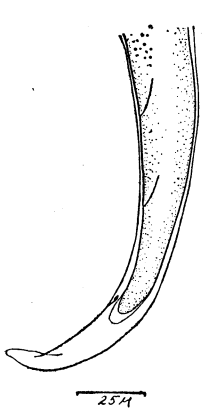

FIGURE 18.—Posterior end of fourth-stage female larva of *Choerostrongylus pudendotectus* showing beginning of last molt. From mesenteric lymph gland of a pig 3 days after experimental infection.

FIFTH-STAGE WORMS

The fourth molt evidently proceeds rapidly, since young fifth-stage worms have been recovered as early as 3 days after experimental

infection. The following descriptions are of a young fifth-stage male recovered from the lungs 5 days after infection (fig. 19) and of a typical female: Male—length, 1.11 mm; width, 45μ; body slender and of same width for most of length; anterior end broadly rounded; esophagus club-shaped, 210μ in length; nerve ring and excretory pore 91μ and 121μ, respectively, from anterior end; spicules rather well formed and filiform, about 418μ in length; bursa and rays present but latter not yet fully developed. Female—body slender, tapering slightly anteriorly and more so posteriorly (fig. 20); esophagus club-shaped, 220μ in length; nerve ring and excretory pore 100μ and 106μ, respectively, from anterior end; vulva anterior to anal opening, 72μ from posterior end; uterine ducts and ovaries fairly well differentiated, the latter extending to a distance of about 197μ anteriorly to vulva; tail slender and digitiform, 45μ in length.

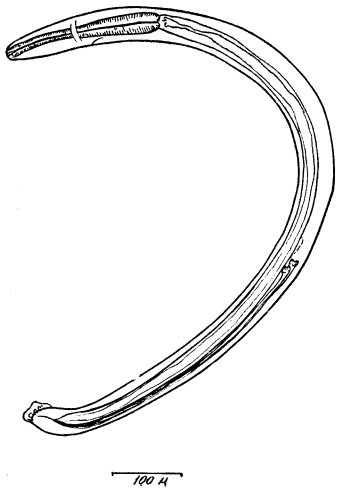

FIGURE 19.—Early fifth-stage male of *Choerostrongylus pudendotectus* from lung of pig 5 days after experimental infection.

DEFINITIVE HOSTS OF SWINE LUNGWORMS OTHER THAN THE PIG

In investigations on the development of lungworms in the definitive host, feeding experiments were conducted not only with pigs, but also with guinea pigs, dogs, a white rat, a monkey, and a cat. As shown in the protocols in this bulletin, the writers developed experimentally *Metastrongylus elongatus* and *Choerostrongylus pudendotectus* in young guinea pigs to a stage in which the uteri of the females contained unsegmented eggs; old guinea pigs were refractory to an infestation with these worms. Von Linstow (*10*) reported a species of lungworm from the bronchi of a dog and called it *Cloacina octodactyla*. Alicata (*2*) noted that the description and figures of *C. octodactyla* were similar to those of *M. elongatus*, and he succeeded in rearing the latter in two 1-month-old dogs as a result of feeding to these host animals several earthworms harboring the infective stage of the lungworm in question. The details of this experiment are given in the protocols. In this connection it is interesting to note that though one dog was killed 32 days after experimental infection, a period adequate for the development of the worms to egg-laying maturity in pigs, the female worms from the canine host contained only nonsegmented eggs in the uteri.

A white rat fed infective larvae of *Metastrongylus elongatus* and *Choerostrongylus pudendotectus* yielded only one larva with the sheaths of the last two molts still present, the larva being found in one of the mesenteric lymph glands. Other larvae possibly present in the lymph glands and elsewhere in the body might have been overlooked in the course of the post-mortem examination of the rat.

An attempt to infect one cat yielded negative results as did also an attempt to infect a primate, the monkey *Macaca lasiotis*.

In addition to parasitizing wild and domesticated swine, *Metastrongylus elongatus* has been reported as a parasite of man in a few instances. Leuckart (*8*) expressed the opinion that human beings acquired an infestation with these parasites as a result of accidentally ingesting an insect or snail which might harbor the parasite in the infective intermediate stage. The Hobmaiers (*7*) found that *M. elongatus* and *Choerostrongylus pudendotectus* failed to develop in the horse.

These data show that, though lungworms of swine are not strictly host specific, they do not develop in other hosts to egg-laying maturity. That dogs are likely to become infested with swine lungworms under favorable conditions may be inferred from the observations made by the senior author that these carnivores sometimes ingest earthworms. Man, on the other hand, is not likely to acquire an infestation with swine lungworms, except on rare occasions, since an earthworm would seldom be ingested by man.

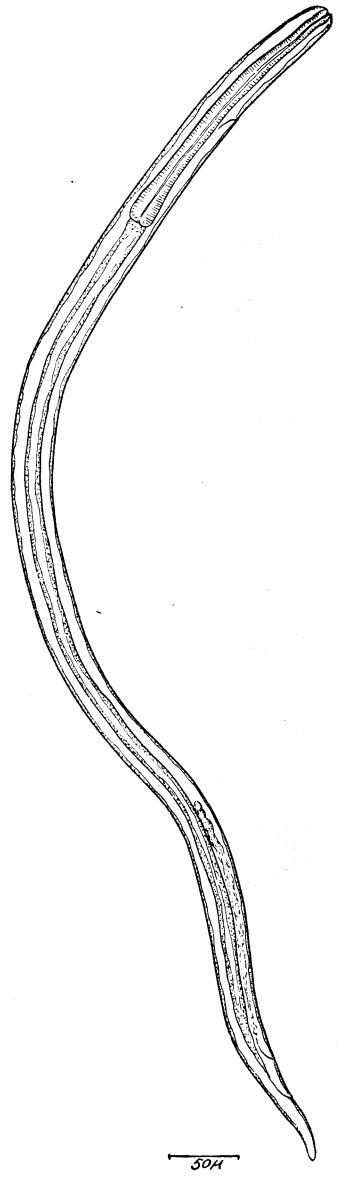

FIGURE 20.—Early fifth-stage female of *Choerostrongylus pudendotectus* from mesenteric lymph gland of pig 3 days after experimental infection.

SYMPTOMS AND LESIONS ASSOCIATED WITH SWINE-LUNGWORM INFESTATIONS

No striking symptoms were observed in pigs experimentally infected with lungworms. This can be explained on the ground that the experimental infections of pigs were light or moderate, apparently insufficient to produce respiratory disturbances. The senior author has observed, however, marked symptoms in young pigs which had acquired a natural infestation with lungworms in a permanent hog lot at the experiment station of the Bureau of Animal Industry at Bethesda, Md. One pig which coughed almost continuously was isolated in a pen having a concrete floor and was observed from day to day. Aside from coughing, the animal showed other respiratory disturbances, such as labored breathing, a symptom suggestive of pneumonia. Post-mortem examination of this pig revealed a heavy infestation with lungworms, most of which, to judge from their size, had not yet attained full growth.

The data given in the protocols with reference to experimental infections of guinea pigs show that these animals became emaciated in some instances and showed respiratory difficulty suggestive of pneumonia. In one case, the animal succumbed 10 days after experimental infection, and in another, 11 days after such infection. Alicata (*2*) noted that 1 of 2 dogs experimentally infected with *Metastrongylus elongatus* died from pneumonia on the nineteenth day after infection. The second dog began to cough about 3 weeks after infection and continued to do so for about a week, at the end of which period the animal was killed and found to be infested with lungworms.

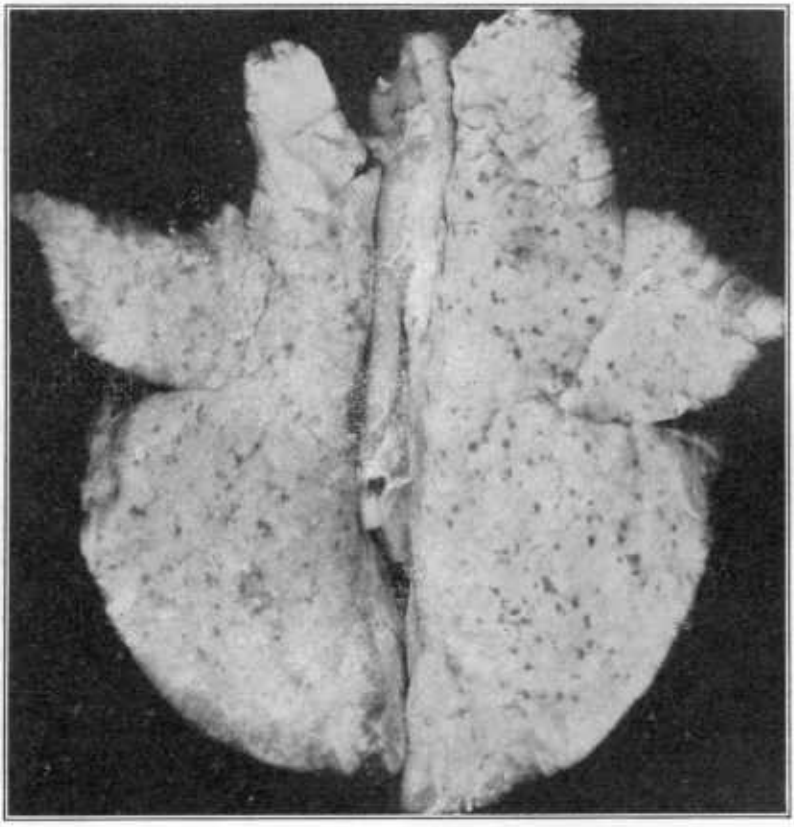

FIGURE 21.—Lungs of a pig 4 days after experimental infection with *Metastrongylus elongatus*. Note petechial hemorrhages.

Early lesions associated with lungworm infestation in pigs, as well as in other experimental animals, are petechial hemorrhages in the lungs (fig. 21), in which lesions lungworm larvae, with the sheaths of the two final molts or with these sheaths already cast off, have been found. The hemorrhages are caused by the penetration of the larvae through the walls of the capillaries, blood oozing into the alveoli as the larvae enter the alveoli and accumulate there. The capillaries in the alveoli have been found to be congested. Lesions found in the lungs of guinea pigs in later stages of development

of lungworms were largely consolidations of portions of the lungs, and emphysematous areas adjacent to consolidated areas. This produced a mottling on the surface of the lungs. Similar lesions have been noted in experimentally infected dogs also (fig. 22) and in pigs experimentally and naturally infected with lungworms (fig. 23), the consolidations marking the locations of the parasites (fig. 24), and being most noticeable in pigs along the posterior borders of the lungs. The bronchi and bronchioles of infested animals contained pus as well as worms.

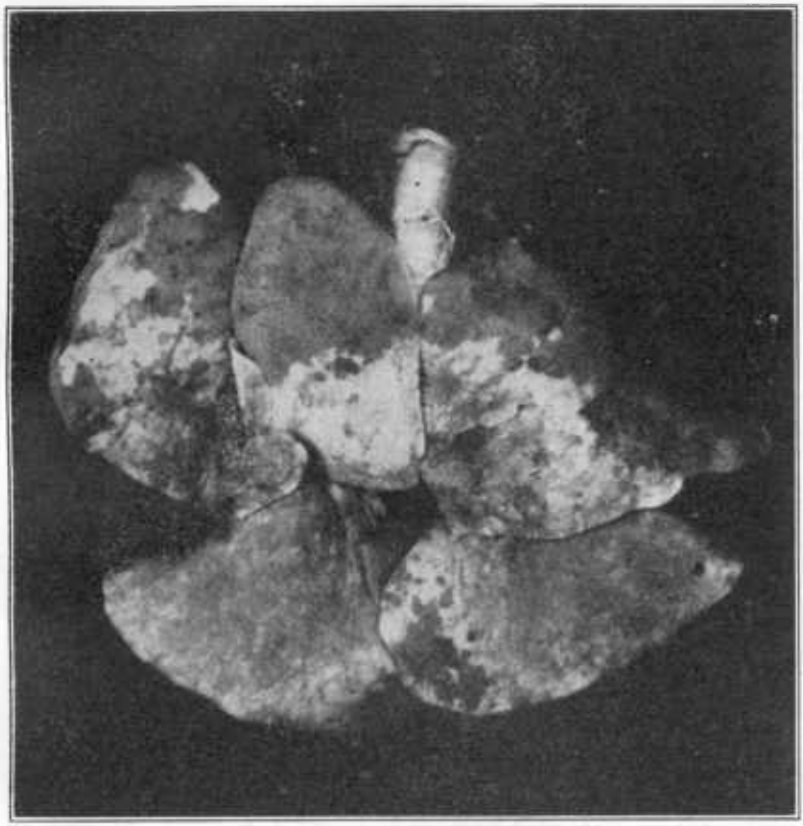

FIGURE 22.—Lungs of a dog 32 days after experimental infection with *Metastrongylus elongatus*. Note mottled appearance. White areas are emphysematous and adjacent darker areas are consolidated. Lungworms were recovered from consolidated portions.

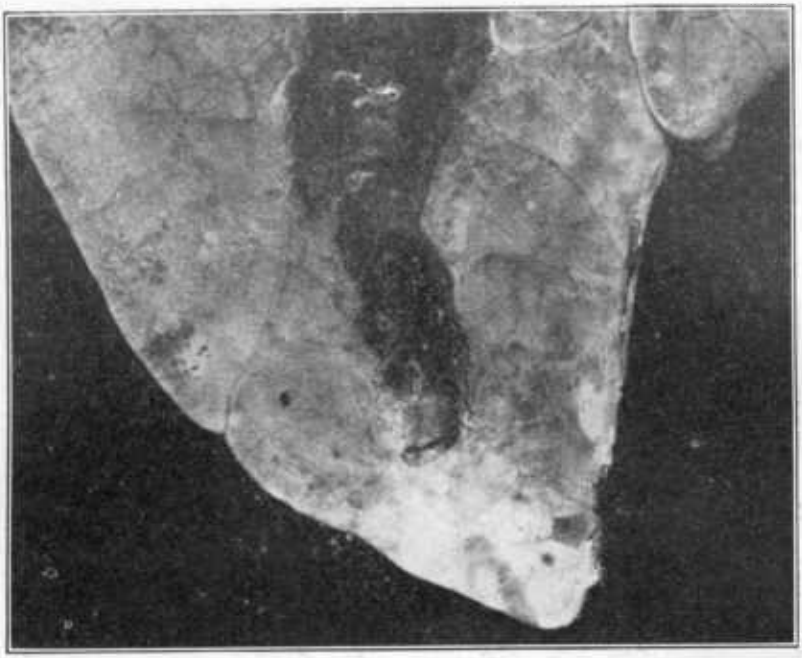

FIGURE 23.—Posterior portion of swine lung 24 days after experimental infection with *Metastrongylus elongatus*. Note emphysematous areas and adjacent consolidations at tip of large lobe.

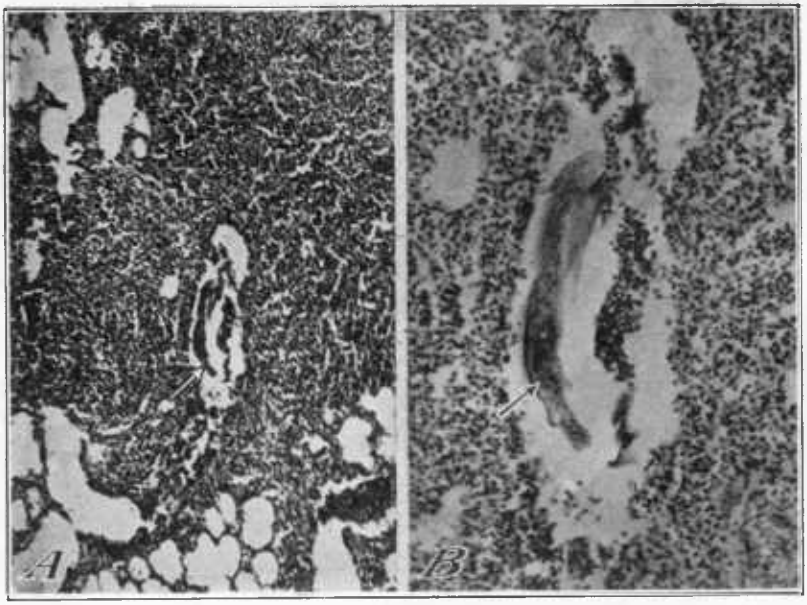

FIGURE 24.—Section of lung of a dog 32 days after experimental infection with *Metastrongylus elongatus*: *A*, Consolidation of lung and section of worm in bronchiole; *B*, greatly magnified view of consolidated portion of lung shown in *A*. Note section of worm and leucocytes in bronchiole.

SUMMARY AND CONCLUSIONS

The three species of lungworms, *Metastrongylus elongatus*, *Choerostrongylus pudendotectus*, and *M. salmi*, parasitic in swine, are heteroxenous nematodes which undergo the first two stages of their development in various species of earthworms.

Three species of the oligochaete family Lumbricidae, namely, *Helodrilus foetidus*, *H. caliginosus* var. *trapezoides* and *Lumbricus terrestris*, one species of the family Megascolecidae, namely, *Diplocardia* sp., and possibly other species of earthworms, have been shown in the course of this investigation to serve as intermediate hosts for swine lungworms.

Earthworms become infested with larvae of swine lungworms as a result of ingesting the eggs eliminated with the feces of infested swine. The eggs are thick shelled and embryonated when passed in swine feces; they do not hatch until they are ingested by the intermediate host. Embryonated eggs obtained from the uteri of gravid female lungworms hatch in vitro if the shell is still thin; the artificially hatched larvae, if fully developed, remain viable in water for several weeks and are infective to earthworms.

The first-stage larvae, free in the lumen of the alimentary canal of earthworms, penetrate the wall of its anterior part and become localized in the posterior half and occasionally only in the anterior portion of the wall of the esophagus, in the wall of the crop, rarely in that of the gizzard, and occasionally in the wall of the portion of the intestine immediately posterior to the gizzard. Sooner or later, they may invade the blood system also and reach the hearts by way of the dorsal blood vessel, in which vessel the larvae become arrested, at least for a time; in the blood system of the earthworm the larvae tend to accumulate in the hearts but rarely succeed in reaching the ventral blood vessel.

In earthworms, growth and development of the larvae to the infective stage are accompanied by two molts; the first molt may take place as early as 8 days after the earthworms have been exposed to infection, but usually a longer period is required; the beginning of the second molt, which becomes evident before the sheath of the first molt has been discarded, has been observed as early as 9 days after exposure of earthworms to infection, but usually a longer period is required. During or after completion of the second molt, the sheath of the first molt is cast off, but that of the second molt is retained by the infective larvae.

Earthworms which were collected in hog lots, pastures, and other places frequented by hogs were found to harbor infestations with lungworm larvae, the locations of the latter corresponding to those observed in experimental infections; the abundance of larvae in natural infestations of earthworms equaled or exceeded that found in experimental infections.

Experimentally, pigs become infected with lungworms as a result of swallowing infested earthworms; under natural conditions pigs become infested with lungworms as a result of swallowing infested earthworms which they bring to the surface in the process of rooting. Although earthworms were readily found in old hog lots rich in humus, in pine woods frequented by pigs, and in other places where feces were allowed to accumulate, they were comparatively scarce in

temporary pastures, and when present in such areas, they were confined mostly to the lower lying portions.

Once they are free in the intestine of pigs, the infective larvae penetrate its wall and follow the course of the lymph. The larvae become arrested in the mesenteric lymph glands at least for a time and are found in these glands in large numbers for a few days after experimental infection. Those larvae that escape from the lymph glands, and sooner or later practically all larvae appear to escape, reach the lungs presumably by way of the right side of the heart.

The worms arrested in the lymph glands, and those which get to the lungs before molting, undergo the two final molts as early as 3 days after entering the definitive host. Further development in the lungs involves growth and differentiation, fully embryonated eggs being present, at least in the species *Metastrongylus elongatus*, as early as 24 days after ingestion of infective larvae.

The most marked pathological changes produced by the developing lungworm larvae are petechial hemorrhages in the lungs, these hemorrhages being rather marked 3 days after ingestion of larvae by pigs or other suitable hosts; larvae with the sheaths of the two final molts have been found in these petechiae. As the worms grow in the lungs, portions of these organs in which the worms accumulate become consolidated, and adjacent areas become emphysematous, giving the affected parts a somewhat mottled appearance. The most outstanding symptoms of lungworm infestation, as observed in the course of this investigation, were a cough and respiratory disturbances.

Metastrongylus elongatus and *Choerostrongylus pudendotectus* are not strictly host specific, since these species were experimentally developed to maturity, though not to egg-laying maturity, in guinea pigs and dogs. In these experimental animals, shelled eggs were found in the uteri of female worms, but the eggs did not develop to a stage that is infective to earthworms. One larva with two sheaths was recovered from a mesenteric lymph gland of a rat to which infective larvae had been administered. A cat and a monkey to which infective larvae were administered escaped infestation.

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